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STUDY OF EXTRATERRESTRIAL LIFE DETECTION CONCEPT

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I. SUMMARY AND INTRODUCTION

The operational effectiveness of any type of extraterrestrial life detection system is dependent upon several key factors, which can be categorized into broad requirements relating to detection methodology. The basic guiding premise relates to primordial Earth life, which is bacterial in nature; it is assumed that lower Martian life forms are also microbial. A new micro-life detection system has been produced from the establishment of the following specific design criteria:

1. The system should have the ability to detect the metabolism of all known culturable Earth bacteria, and, further, should not be restricted to a single nutrient-milieu that may be presented to all given indigenous species.

2. The detector element(s) should have the ability to obtain metabolic signals from minimum concentrations of bacterial life forms and to categorize them in such detail that the type(s) of life processes on Mars could be ascertained.

3. The system should allow continued research on duplicate Earth-located laboratory models, after any positive detection signals have been transmitted from Mars to better clarify the findings in terms of a possible foreign bio-chemistry.

4. The system should have a sufficient number of subminiaturized bio-detector elements of such operational reliability that any failures of a reasonable percentage of these components will not greatly harm the statistical results of the life detector system.

5. The system must have very low power requirements with high accuracy to assure continued life detection for extended periods of time in the event that Martian bacterial species may be characterized by minute metabolic rates (USAF School of Aerospace Medicine, 1962). The system must have built-in controls and/or a capability that will establish complete validity of any bio-detector test element signals.

6. The bio-detector system should not require gravity-oriented positioning in the event that soft landing encounters uneven terrain with the possibility of upsetting the system's normal operating position.

7. The system must be small, compact and low weight, and above all, be within the existing state-of-the-art to assure rapid hardware development.

The above requirements have led to investigation of a concept, "MARBAC", based upon the detection and identification of microorganisms as a result of their metabolism. Metabolic reactions result in changes in the compositions of appropriate nutrients; changes in the compositions of nutrients are reflected in changes in the oxidation-reduction potential between electrodes in the nutrient solution. The laboratory test results and design studies indicated that all of the above requirements could be attained.

An important feature of MARBAC is the large number of individual metabolism-detection cells that can be located within a small volume. This feature offers the unique possiblility for the testing on Mars of many nutrient-milieus covering a wide range of temperature, light, acidity, and osmotic and nutritional conditions in one small, compact unit.

The purpose of this investigation was to conduct intensive laboratory research tests of the MARBAC detector cell concept to evaluate signal response time and level for all major categories of culturable, earth bacteria, including systematic parametric studies of nutrients and environmental factors.

Results of the oxidation-reduction potential studies conducted under this contract have verified the hypothesis that such techniques can be used to detect the presence of bacteria. The use of changes of oxidation-reduction potentials to detect microbial metabolism and growth has been demonstrated in various types of microorganism-nutrient systems. The effects of certain primary variables, such as pH, temperature, concentrations and types of microorganisms and nutrients, growth factors, and gaseous environment were investigated, for the purpose of increasing the extent and rates of changes of oxidation-reduction potentials.

As a result of the experiments conducted under this contract, correlations were developed between oxidation-reduction potentials and bacterial counts. The reproducibility of potential readings was acceptable,

and was within a 20 millivolt range under favorable conditions. A broad spectrum of desert soils, such as those that might be found on Mars, was inoculated into trypticase soy broth; potential changes as high as 690 millivolts were obtained, due to the metabolism and growth of microorganisms indigenous to the soils. Studies of the effects of miniaturizing the test cells indicated that experimental cells containing 0.4 milliliter of nutrient and microorganisms could be operated to produce essentially the same changes in potential as larger cells containing 31 milliliters of solution and microorganisms.

II. DISCUSSION OF EXPERIMENTAL APPARATUS AND TECHNIQUES

A. Equipment and Apparatus

A typical laboratory test setup, with the related instrumentation (including the digital voltmeter), is shown schematically in Figure 1.

A 50-channel automatic recording, digital voltmeter system was employed early in this program for data acquisition. This system provided automatic readings at 30-minute intervals. Another limitation of this voltmeter was found to be its input impedance, which was approximately 10^7 ohms. It was known that precise measurements of the oxidation-reduction potential in relatively dilute, small volume experimental cells employed in the studies conducted under this program would have to be accomplished with a minimum cell current drain.

In order to provide a digital voltmeter system with more desirable features than those described above, a Dymec Model DY-2010F automatic card recording system was installed. A comparison of the two digital voltmeter systems is presented below:

System	New	Old
Input impedance (ohms)	10^9	10^7
Time required for reading (sec)	0.1	3
No. of channels available	300	50
Time between cycles	1 sec.to 30 min.	30 min., fixed
Method of data printout	Punched cards	Tape

A photograph of the newer digital voltmeter and key punch data printout system is presented in Figure 2.

An illustration of the effects of high and low impedance in voltmeters on the oxidation-reduction potentials is shown below. The medium tested was sterile trypticase soy broth (described in Table I). Readings were obtained manually at intervals of two seconds, and the sampling period was one second.

Voltmeter Impedance (Megohms)	Oxidation-Reduction Potential, Platinum vs. Saturated Calomel (mv.)									
	1	2	3	4	5	6	7	8	9	10
10	139	131	125	122	120	118	117	115	114	113
10,000	146	147	147	147	147	147	148	148	147	148

The above data indicate that in studies where the poising of the medium is small, the amount of current flowing through the test cell may be sufficient to affect the reading of a single potential, even under essentially "open circuit" conditions.

Table II shows a typical study of the reproducibility of results. Each channel was tested eight times consecutively, with five seconds between tests. The 0.1 mv. reading is given, although in microbiological testing only millivolts are considered significant. In Table II, channels 7 and 8 show a significant change compared to channels 1 through 6; channels 7 and 8 contained nitrate broth while other channels contained nutrient broth (5 and 6), trypticase soy broth (3 and 4), and synthetic medium C (1 and 2).

In a series of studies with uninoculated, nonagitated solutions in typical test setups, variations in potential readings were not greater than ± 5 millivolts over any two-hour period with readings 1/2 hour apart. These variations appeared mostly caused by medium and atmospheric factors. The voltmeter (Dymec Model DY-2010F) contains internal voltage standards against which the machine may be calibrated at any time; the standards are for ± 1.00000 and 0.00000 volts. The machine is typically calibrated below 0.1 millivolt, frequently to within ± 0.01 millivolt.

The general reproducibility of potential readings from identical test setups with the new voltmeter is illustrated by the curves in Figure 3. More than 70 percent of the voltmeter readings produced

curves similar to those of brain heart infusion. More than 90 percent of the curves for identical tests showed similar initial (uninoculated) and final (full growth) redox values for any given medium; the starting and final potentials were usually within a 20 millivolt range.

Coulter Counter: A Coulter Counter was employed for counting bacterial particles in many of the experiments described in this report.

The changes in bacterial count concurrent with redox changes were ascertained frequently, to attain a basic understanding and control of the bio-electrochemical processes.

Standard plate counts are known to be very time consuming, and immediate results were desired. Turbidimetric procedures and direct microscopic counts were also unsatisfactory because of their limited accuracies. Automated accurate counting methods at all inoculum dilutions, such as those obtainable with the Coulter Counter, were therefore adopted. A schematic diagram of this apparatus is shown in Figure 4.

Fused manometers were employed with the Coulter Counter instead of the cemented orifices ordinarily used, to permit more rigorous cleaning of the 30 micron aperture through which the bacterial suspension flows.

The addition of detergents to bacterial solutions was investigated for the combined purposes of decreasing the clogging of the aperture, decreasing bacterial clumping or increasing dispersion, or for decreasing bacterial conductivity as an aid to counting.

Several calibration procedures were necessary to attain maximum accuracy. These included aperture current and voltage polarization after a counting cycle (typically shown in Figures 5 and 6), determination of the 0.05 milliliter nominal manometer volume to three significant figures, determination of background count, and establishment of the proper threshold level for the greatest counting accuracy.

The following typical Coulter Counter test and results may be compared to other counting techniques, and indicate the applicability of this instrument to microbiological research. Twenty-five milliliters of an 18-hour nutrient broth culture of Escherichia coli was sedimented at 8200 g's. The cells were washed in saline solution and centrifuged again. They were resuspended in exactly 25.0 milliliters of saline solution. The suspension was then diluted 1:1000; 8:100,000; 6:100,000; 4:100,000; and 2:100,000; these dilutions represent ratios of 1:2:3:4:50.

The test results, tabulated below, were corrected for the particle count of the saline blank:

Dilution Ratio of Original Sample	Counter Counts in 0.0528 ml. Diluted Sample	Counts per ml. Converted to Original Culture	% Deviation From Mean
1:50,000	469	4.44×10^8	0.11
1:25,000	900	4.26×10^8	3.95
1:16,670	1,401	4.42×10^8	0.33
* 1:12,500	2,835	6.71×10^8	--
1:1,000	24,413	4.62×10^8	4.16

Mean = 4.435×10^8

* This dilution was not included in the mean due to an obvious but unexplained error.

The percent deviation from the mean was quite acceptable in this test, in comparison with other counting techniques.

Electrodes: The continual calibration and maintenance of the platinum and the saturated calomel electrodes were known to be of vital importance, as factors in obtaining consistent quantitative redox results. A seven-step checkout procedure was adopted for electrode preparation prior to the initiation of a formal experiment. These steps are summarized in Table III.

The electrode system used in the earlier phases of this program included conventional calomel and platinum button electrodes. These electrodes were disinfected by immersion in mercuric chloride solution.

This electrode system was later modified to permit sterilization by autoclaving. The conventional saturated calomel electrodes were connected to saline salt bridges (0.9 percent sodium chloride by weight, in Luggin capillaries), and the platinum electrodes were in the form of wires coated with chemically resistant paint so that only the tips were exposed. The saline solution was used instead of saturated potassium chloride solution to retard diffusion. The excellent reproducibility that was attained with these electrodes is shown in Figure 7, and the effect of various salt bridges is shown in Figure 8.

B. Microbiological Techniques

1. Sterility Studies

When it had been demonstrated that an acceptable level of reproducibility of potentials had been attained with the electrode system employing saturated calomel reference electrodes with saline solution salt bridges and platinum wire electrodes (see Figure 7), life tests of the various microorganism-nutrient systems were initiated.

Twelve sterile cells were assembled, six with trypticase soy broth and six with thioglycollate broth. These cells were allowed to stand until evidence of contamination was obtained, both by visual inspection (turbidity) and change of potential. The six cells containing thioglycollate broth were still sterile after 45 hours, but two of the six cells containing trypticase soy broth became contaminated after 33 to 34 hours. The significant factor is that contamination could be detected by the potential change.

In another study for sterility, the test was terminated after 135 hours, during which time no contamination had occurred in eleven cells, but one cell had become contaminated after the first 44 hours. Lack of contamination in the eleven cells was verified by placing 0.5 milliliter from each cell on trypticase soy agar plates and incubating at 37°C; no growth occurred. It was concluded that the experimental apparatus used in these studies could be maintained in a sterile condition for extended periods of time.

2. Correlations Between Coulter Counter, Bacteria Plate, and Turbidimetric Counts

Figure 9 shows typical results of routine serial dilution plate counts utilizing agar overlay and pour plate techniques. The variability of counts in this method is considerable, and the loss of accuracy in many instances would negate the use of the method in precise redox work.

The overlay plates were made by placing one milliliter of an organism sample on top of 15 milliliters of solidified agar medium in a Petri dish, pouring 2 milliliters of molten medium on top of the agar, and mixing. The overlay method appeared to provide better counts than pour plates, especially by making the colonies more visible and readily enumerated.

The pour plates were prepared by placing one milliliter of a dilution in an empty sterile Petri dish and then mixing with approximately 15 milliliters of sterile, molten (about 44°C) medium.

Wilson and Miles[1] discussed the "experimental errors of considerable magnitude" that could be produced by typical plate count methods and, according to another reference[2], the standard plate counts may be subject to the following errors:

(a) Variations in the number of individual cells per clump

(b) Failure of some bacteria to form visible colonies because of unfavorable composition or reaction of medium, unfavorable oxygen tension, or unsuitable incubation temperature

Turbidimetric techniques are useful for heavy concentrations of microbes (about 10^8 cells per milliliter and higher); the effect of dilution of an inoculum is shown in Figure 10. Turbidity is also useful in obtaining relative values of microbial growth, provided the growth is heavy. The technique cannot be employed with smaller numbers of microbes without the development of specialized instrumentation, and therefore had only a limited use in the MARBAC program.

Under appropriate testing conditions, precision limits were established for plate (viable) and Coulter (electronic) counts of bacteria (Table IV). In plate counts, the percent deviation from the mean, for either of two technicians counting plates twice, was approximately 6 percent. The lowest count four plates and the highest count four plates (of the total 20 plates) gave 11 percent deviation from the mean. Four plates were routinely prepared for each dilution of microbes during a redox study.

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1. Wilson, G.S., and Miles, A.A., Topley and Wilson's Principles of Bacteriology and Immunity, 3rd ed., Williams and Wilkins Co., Baltimore. (1946)

2. American Public Health Association, Inc., Standard Methods for the Examination of Dairy Products; Microbiological and Chemical, 11th ed., American Public Health Association, Inc., New York. (1960)

In Coulter counting, the average percent deviation from the median was 3 and 4 percent for two studies. The lowest four of 20 counts provided 6 and 7 percent deviations from the median, while the highest four counts had 3 and 8 percent deviations from the median.

III. DATA AND RESULTS

A. Evaluation of Control Systems

An experiment was conducted to determine the stability of oxidation-reduction potentials of the control media used in the tests described in this report, to verify that the potential changes obtained by inoculation with various microbes was actually the result of such inoculation.

The media tested included the following:

(1) 0.9% saline solution (a nongrowth medium), sterile

(2) 0.9% saline solution, lightly inoculated with Escherichia coli (10^7 cells in 31 milliliters)

(3) 0.9% saline solution, heavily inoculated with Escherichia coli (10^9 cells in 31 milliliters)

(4) Aerobic growth media, or complex synthetic (nutrient broth, brain heart infusion, and synthetic medium C), sterile

(5) Anaerobic growth medium (thioglycollate broth), sterile

(6) Inoculated medium (Escherichia coli) without growth

(7) Inoculated medium (Escherichia coli)

(8) Growth medium that became contaminated

The results are shown in Figures 11 and 12. It was found that the control systems were reproducible without $\pm$ 20 millivolts, and were stable for periods exceeding 10 hours. Potential changes occurred only in those media that were not sterile and in which the microbes could grow.

B. Oxidation-Reduction Potentials Obtained from Inoculated Media

Escherichia coli: This microorganism was chosen as the primary test organism in this program because it has been the subject of more extensive metabolic research than any other microorganism. The effects of various modified growth media and growth factors upon the variation of the oxidation-reduction potential with time were examined, and tests were conducted on the redox stability and reproducibility of sterilized controls.

The effects of basic media, as well as modifications in ingredient ratios, were evaluated. The various media are described in Table I.

In each experiment, the inoculum was prepared as follows:

(1) Inoculum growth: Eighteen hours growth from a one-loopful inoculum in a medium similar to that used in a specified test.

(2) Inoculum preparation: Cells washed, sedimented by centrifugation at 8200-g for ten minutes, resuspended in 0.9 precent saline, centrifuged and resuspended again.

Changes of potential produced by Escherichia coli growing in six test media are presented in Figure 13, wherein the similarity of the redox behavior of four media are evident, and the special behavior of nitrate broth and thioglycollate broth are shown. The inoculum concentration was the same in all media (3×10^6 organisms per milliliter), and the conditions were ambient. Grouping of media with respect to redox changes was an initial step towards providing a biochemical basis for the changes.

In another experiment, Escherichia coli at a concentration of 10^9 cells in 31 milliliters was inoculated into three nutrients at ambient conditions. The nutrients included thioglycollate broth, brain heart infusion, and nutrient broth (all of which are described in Table I). Brain heart infusion and nutrient broth had initial potentials of +200 millivolts, whereas that of thioglycollate broth was -150 millivolts. A significant change was rapidly produced by thioglycollate broth, after

inoculation (300 millivolts in approximately 1-1/3rd hour), while potential changes of 600 millivolts were produced in brain heart infusion in 3-1/2 hours and by nutrient broth in 7 hours. These results are shown in Figure 14.

Pseudomonas aerugenosa: The oxidation-reduction potential changes produced by Pseudomonas aerugenosa in brain heart infusion and in nutrient broth (described in Table I) are shown in Figure 15. The inoculum concentration was approximately 10^8 organisms in 31 milliliters. A potential change of approximately 170 millivolts was produced in both media in 10 hours.

Bacillus subtilis: The oxidation-reduction potential changes produced by Bacillus subtilis in brain heart infusion and in nutrient broth (described in Table I), are also shown in Figure 15. The inoculum concentration was approximately 10^8 organisms in 31 milliliters. A potential change of approximately 280 millivolts was produced in both media in 12 hours.

B. subtilis was tested in a variety of media in which the concentration of inoculum was 10^6 microorganisms per milliliter; the cutoff time was 16 hours, by which time the potentials had become constant. The maximum changes in potential are tabulated below:

Medium	Potential Change (Millivolts)
Thioglycollate broth	0
Synthetic Medium C	0
Nutrient Broth	175
Nitrate Broth	300
Brain Heart Infusion	400
Trypticase Soy Broth	400

Alcaligenes faecalis: This microorganism was tested in a variety of media. The inocula were approximately 10^6 microbes per milliliter, and the cutoff time for all tests was 16 hours, which was the full time required for the potential changes listed below:

Medium	Potential Change (Millivolts)
Thioglycollate Broth	0
Synthetic Medium C	75
Nutrient Broth	250
Nitrate Broth	200
Brain Heart Infusion	300
Trypticase Soy Broth	300

Other Organisms: The potential changes obtained by inoculating trypticase soy broth (described in Table I) with various types of bacteria were studied. Test conditions were aerobic at room temperature. All inoculum concentrations were not the same, which would have some influence on the rate of change of the potential, but all had been incubated at 37°C for more than 18 hours, so that the inocula were reasonably concentrated at the time of inoculation.

The extent and rate of potential changes obtained after inoculation with Alcaligenes faecalis, Erwinia caratovora, and Aerobacter aerogenes are shown in Figure 16; Bacillis cereus, Bacillus subtilis, and Pseudomonas fluorescens in Figure 17; and Bacillus megaterium, Streptococcus faecalis, and Serratia marcescens in Figure 18. Each curve is an average of three tests.

When the above nine microorganisms are compared, it is apparent that the greatest and most rapid change of potential was obtained with Aerobacter aerogenes, followed by Erwinia caratovora. However, all of the microorganisms produced changes exceeding 200 millivolts in less than 20 hours.

The tests also indicated that the shape of the curve reflects the nature of the metabolic reaction; for example, Pseudomonas fluorescens, which is an aerobe, produces a relatively rapid change in potential initially, but reaches a plateau after a total change of only 200 millivolts because the potential has become too reduced for further growth; in contrast, Alcaligenes aerogenes, which is a facultative anaerobe, can grow at potentials that are too reducing for some other types of microorganisms and so is able to produce a 600 millvolt change.

A comparison is presented in Figure 19, showing the total potential changes, and the times required to produce the changes, of six media after inoculation with each of four types of microorganisms. One distinctive difference between microbes was shown by the 300 millivolt total potential change produced by Escherichia coli in nitrate broth compared to the 750 millivolt change produced by Alcaligenes aerogenes. Both organisms have been established as changing nitrate into nitrite, by other investigators. The redox potential provides a differential feature with regard to the behavior of these microorganisms in a nitrate broth. This is just one example of the use of the redox potential for distinguishing between microbes inoculated into the same medium.

Soil Samples: Twelve soil samples were obtained from Dr. Roy Cameron (Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California). These samples represented a reasonably broad spectrum of soils, and were obtained from diverse geographical locations and at various depths; most samples were dried, but some contained residual moisture. Samples of these soils were inoculated into trypticase soy broth, and the oxidation-reduction potentials produced by the indigenous microorganisms in these soil-nutrient systems were determined. The soil samples are described in Table V.

One of the soil samples (No. 4) was added aseptically to sterile, deionized water (1 gram soil in 10 milliliters of water) and shaken by hand for ten minutes. Portions of this solution were then inoculated into trypticase soy broth and thioglycollate broth (described in Table I) in the proportions of 1 milliliter soil solution in 30 milliliters of nutrient. The experiment was run in triplicate.

Two of the test cells inoculated into trypticase soy broth showed satisfactory reproducibility. The potentials began to change approximately 7 hours after inoculation, and the time of the most rapid change of potential was separated by only one-half hour for the two cells; the total potential change was in the range of approximately 215 to 300 millivolts. The third cell produced a similar potential change (approximately 280 millivolts), but the change occurred after a longer period (13 instead of 7 hours). There was some question concerning possible initial contamination of the two cells that produced the more rapid potential change, since two control (uninoculated) cells also produced potential changes within eight hours after inoculation of the test cells. Therefore, this experiment was essentially repeated.

Another sample of the same soil was used in the repeat experiment, but only the liquid solution and extremely small particles of the soil were used. In this experiment, the changes in potential occurred 11 to 15 hours after inoculation. The potential change occurred in two phases. The first was a change of approximately 250 millivolts that occurred during the first 18 hours after inoculation; the second was a change of an additional 150 to 300 millivolts, which was still changing when the experiment was terminated approximately 40 hours after inoculation. These results are shown in Figure 20.

In two additional experiments, soil samples were inoculated into trypticase soy broth in the proportion of 0.1 gram soil in 30 milliliters of nutrient. The results of one experiment are summarized below and are shown in Figure 21.

Soil	Time Required to Initiate Change in Potential (hours after inoculation)	Total Change in Potential (mv)
9	19	500
2	19	400
1	16	470 *

* Potential was still changing after 52 hours.

The results of the other experiment are shown in Figure 22 and summarized below:

Soil	Time Required to Initiate Change in Potential (hours after inoculation)	Total Change in Potential (mv)
3	10	690
5	24	610
6	18	615
9	20	530

In each case, the electrodes and media were sterilized by autoclaving. Three test cells were inoculated with each soil, and three were uninoculated controls.

A portion (0.5 milliliter) of the trypticase soy broth of each test cell was plated on trypticase soy agar before inoculation, and incubated at room temperature, in order to assure that there was no contamination before inoculation.

In still another experiment (No. 110), garden soil was inoculated into selective media. The sample was obtained from the top 1-1/2 inch of surface soil, and contained some undecomposed manure. The basal selective medium is described in Table I. Enrichment conditions for various micro-organisms in the selective media are described below:

Azotobacter: Ethyl alcohol (4.0 grams per liter) added to basal selective medium described in Table I; atmosphereic N_2 as sole nitrogen source; incubated at 25-30°C without illumination, aerobic; pH 7.9 (should be 7.0).

Nitrosomonas: NH_4Cl (1.5 grams per liter) and $CaCO_3$ (5.0 grams per liter) added to basal selective medium described in Table I; incubated at 25-30°C without illumination, aerobic; pH 7.5 (should be 8.5).

Nitrobacter: $NaNO_2$ (3.0 grams per liter) added to basal selective medium described in Table I; incubated at 25-30°C without illumination, aerobic; pH 7.8 (should be 8.5).

Thiobacillus: NH_4Cl (1.0 gram per liter) and $Na_2S_2O_3.7H_2O$ (7.0 grams per liter) added to basal selective medium described in Table I; incubated at 25-30°C without illumination, aerobic; pH 7.6 (should be 7.0).

The results indicated that Azotobacter grew, followed by secondary growth of some other organism. Apparently there was no growth of Nitrosomonas or Thiobacillus. A change of approximately 110 millivolts in potential indicated possible growth of Nitrobacter.

Another sample of the garden soil was inoculated into the medium selective for Azotobacter. Azotobacter in this medium produced a change in potential, which was increased by further addition of ethyl alcohol.

When the garden soil was inoculated into the medium selective for Azotobacter, the potential changed over a 100 hour period as shown in Figure 23. The potential drop apparently occurred in two steps, possibly either because of metabolism and growth of a second (unknown) type of organism in the soil, or because of a temporary availability of oxygen to the solution.

C. Studies of Effects of Variables

1. Concentrations of Media

Experiments were conducted to study the effect of varying the concentration of the medium upon the oxidation-reduction potential obtained after inoculation with Escherichia coli. In one experiment, the concentration of Synthetic Medium C (described in Table I) was varied from full strength to a dilution of five times the original volume. The inoculum concentration was approximately 4×10^9 organisms in 31 milliliters, and dilutions of the medium were made with 0.9 percent saline solution. The results (see Figure 24) showed that concentrations of the medium from full strength to a dilution of four times the original volume had essentially no effect upon the change of potential; dilution to five times the original volume resulted in a decrease in total potential change, to 200 millivolts instead of the change of approximately 700 millivolts obtained with the more concentrated solutions.

In similar experiments, the concentration of nutrient broth was varied from double strength to half strength. The inoculum sizes were approximately 2×10^9 and 10^9 microorganisms in 31 milliliters. Dilution to full strength was made with distilled water, while further dilution to half strength was made with 0.9 percent saline solution. The results are presented in Figures 25 and 26. When the inoculum size was 2×10^9 microorganisms in 31 milliliters, a potential change of approximately 600 millivolts was obtained in four to seven hours, and there was apparently no significant effect of dilution of the medium. When the inoculum size was 10^9 microorganisms in 31 milliliters, there was an effect of dilution upon the total change of potential; the double strength solution resulted in the greatest total change of potential; the time required to produce a given change of potential apparently was less for the more concentrated solution.

In another experiment, the concentration of nutrient broth (described in Table I) was varied from a dilution of 1:2 to 1:3.5, and the inoculum concentration was 10^8 cells in 31 milliliters. The results are shown in Figure 27. Again, it was shown that the most dilute media required the longest time to produce a given change of potential.

In another experiment, the concentration of nutrient broth (described in Table I) was varied from one-sixteenth to full strength. The inoculum concentration was approximately 10^7 cells in 31 milliliters, and the conditions were ambient. The results are shown in Figure 28. It was found that the use of half-strength media resulted in a more rapid change of potential than in the case of full-strength media (13 hours in comparison to 20 hours), but one-fourth strength (or more dilute) media extended the length of time required to produce a change of potential.

2. Comparison of Aerobic and Anaerobic Conditions

The effect of atmospheric conditions on the potential change produced by Escherichia coli was determined in nutrient broth as well as in 0.9 percent saline solution; the concentration of the nutrient broth was one-half that described in Table I. The inoculum concentration was approximately 10^9 cells in 31 milliliters, and the temperature was ambient. The results are shown in Figure 29, where it is seen that changes from aerobic to anaerobic conditions did not significantly affect the total potential change of Escherichia coli in nutrient broth; no potential change was produced in saline solution, as was anticipated, since there is no nutrient in such a solution.

Another experiment was conducted to compare the changes in potential caused by microorganisms under aerobic and anaerobic conditions. The microorganisms included Escherichia coli and Clostridium sporogenes, at a concentration of approximately 10^7 microorganisms per milliliter. The media were trypticase soy broth and thioglycollate broth. Anaerobiosis was accomplished chemically (by the use of thioglycollate broth) or physically (by bubbling trypticase soy broth with helium). The results of this experiment are shown in Figure 30.

Escherichia coli in trypticase soy broth bubbled with helium produced a significant change in potential within a half hour after inoculation; the total potential change of approximately 600 millivolts (average of two samples) was complete within five hours after inoculation. During the period following this change of potential (approximately 15 hours), the potential rose approximately 125 millivolts. This rise of potential was caused by a leakage of air (oxygen) through the filter, as determined by using methylene blue as an oxidation-reduction indicator. The results of the experiment were not nullified by this leakage, because the leakage occurred after the maximum change in potential.

Escherichia coli in thioglycollate broth produced a total change in potential of approximately 175 millivolts. This change occurred within 10 hours after inoculation. The potential then rose slightly, possibly because the thioglycollate broth was unable to maintain the reduced condition.

Clostridium sporogenes in thioglycollate broth produced a change in potential of 250 millivolts, which required 22 hours. The same microorganism in trypticase soy broth bubbled with helium produced a change of 640 millivolts within 16 hours after inoculation.

The results of these experiments indicate that there is little effect of the atmosphere (aerobic vs. anaerobic) upon the potential change produced by inoculating nutrient broth with Escherichia coli at high levels of concentration of the microorganisms (10^9 cells per milliliter). A greater and more rapid potential change is produced by inoculating trypticase soy broth with Escherichia coli than by inoculating thioglycollate broth with that organism at an inoculation concentration of 10^7 cells per milliliter; these data indicate that the physical removal of oxygen from the medium (by bubbling with an inert gas) is more effective than chemical removal. The same effect was noted with Clostridium sporogenes. It was further noted that the total potential change obtained by inoculating trypticase soy broth with Clostridium sporogenes, an anaerobe, was essentially the same as that obtained by inoculating the same medium with Escherichia coli, an aerobic (facultatively anaerobic) organism, although the potential change with the latter was more rapid.

3. Modifications of Media

Concentration of Glucose: The effect of the amount of glucose (0.0001% to 5.0%) on the oxidation-reduction potential was investigated. The medium was synthetic medium C (described in Table I). The organism was Escherichia coli, and the concentration of the inoculum varied from 10^9 to 4×10^9 organisms in 31 milliliters. The results are presented in Figures 31, 32, and 33. At a particular concentration of organisms, the 1 percent concentration of glucose seemed to be the most effective in producing a rapid change of potential. The differences in the curves for 0.1 percent glucose at the various bacterial concentrations may be due to differences in the total amount of endogenous metabolism; the greatest potential change was obtained with a concentration of 0.1 percent glucose when the inoculum concentration was 2×10^9 organisms in 31 milliliters or higher.

Addition of glucose to trypticase soy broth, above the normal concentration specified in Table I, resulted in three apparent differences in the oxidation-reduction potential changes ordinarily obtained after inoculating trypticase soy broth with Escherichia coli: (a) In the initial oxidation-reduction potential; (b) in the total potential change; and (c) in the shapes obtained by plotting potential versus time.

The starting potential of the trypticase soy broth with additional glucose was approximately 100 millivolts less than that of the unmodified medium. Since the minimum potentials were essentially the same in both the medium containing the additional glucose and the one that did not, the total potential change in the medium that contained the additional glucose was approximately 100 millivolts less than in the one that contained only the usual amount of glucose. After the maximum potential changes had been reached in both media, the potential of the medium containing additional glucose rose more than 100 millivolts higher than that of the medium that contained only the usual amount of glucose; this rise in potential may have been caused by a continued increase in the acidity of the medium containing additional glucose.

Concentration of Thioglycollate: The effect of the concentration of thioglycollate (0 to 0.5%) on the oxidation-reduction potential was investigated. The medium was nutrient broth (described in Table I). The organism was Escherichia coli, and the inoculum concentration was approximately 10^{10} organisms in 31 milliliters. The results are presented in Figure 34. The addition of thioglycollate to the nutrient broth lowered the initial (uninoculated) oxidation-reduction potential, and also resulted in a smaller total change of potential. Thioglycollate apparently poised the potential, so that the greatest and most rapid change of potential was obtained with a medium containing no thioglycollate.

Sodium thioglycollate was also added to Synthetic Medium C (described in Table I) in concentrations up to 0.2 percent, to determine the effect of thioglycollate concentration on the potential obtained in the medium. It was found that the uninoculated potential was lowered approximately 300 millivolts with the addition of sodium thioglycollate over this concentration range (see Figure 35).

It may be concluded from these data that the addition of thioglycollate to either nutrient broth or Synthetic Medium C results in a lower uninoculated potential and less total change of potential, and that these effects are greater as more thioglycollate is added. Therefore, from the viewpoint of enhancing oxidation-reduction potential changes, thioglycollate should be omitted from the media.

Concentration of Potassium Nitrate: The effects of potassium nitrate concentration on potential changes produced by Escherichia coli in nutrient broth were studied. Three concentrations (0.1, 0.05 and 0.01%) of potassium nitrate were used; three flasks of each concentration were tested, and there were three controls (nutrient broths without nitrate). Inocula were approximately 3×10^6 organisms per milliliter.

The results, presented in Figure 36, demonstrate that the addition of potassium nitrate to nutient broth, even at concentrations as low as 0.05 percent, greatly inhibited the potential change. At the lower (0.01%) concentration, the potential changes were similar to those of nutrient broth alone, but differed by being about one hour slower in reaching the maximum change in potential, and by rising thereafter over 100 millivolts from the lowest potential. The broad range (100 to 150 millivolts) in which the potential changes occurred for the six flasks with 0.1 percent and 0.05 percent potassium nitrate was typical of many tests with nitrate.

Concentration of Phosphate Compounds: Attempts were made to study the effects of phosphate compounds upon the poising ability of a medium. The medium chosen was Synthetic Medium C. Potentials obtained from solutions of the individual constituents in distilled water, but at the proper concentrations prescribed for this medium, are presented in Table VI.

The constituents were then combined in the proper proportions for Synthetic Medium C, except for the phosphate compounds (KH_2PO_4 and Na_2HPO_4); the potentials obtained from these solutions are also given in Table VI. The addition of Na_2HPO_4 lowered the potential of the solution, and the potential of the solution was roughly inversely proportional to the concentration of the salt; the addition of KH_2PO_4 had no effect on the potential.

As a result of these data, Synthetic Medium C was modified to form a medium which would be changed in potential soon after inoculation. The modification was accomplished by increasing the concentration of glucose from 0.1 percent (1 gram per liter) to 1.0 percent,

and the Na_2HPO_4 was reduced to one-tenth the standard concentration (the composition of Modified Synthetic Medium C is described in Table I). The media were inoculated with Escherichia coli at a concentration of approximately 2 X 10^{10} organisms in 31 millileters, and the atmosphere and temperature were ambient.

The results of this experiment are shown in Figure 37. Although the modification of the medium changed the initial potential, it did not affect the total change of potential after inoculation, or the time required to obtain that change.

The experiment was repeated, but at a lower inoculum concentration (10^8 organisms in 31 milliliters). The results are shown in Figure 38, where it may be seen that there was no definite effect of modification of the medium on either the total change of potential, or on the time required to obtain the change (the lines in Figure 38 crossed). The rate of the change of potential produced in the modified medium may have been greater initially (that is, during the first eight hours after inoculation) in the modified medium.

Concentration of Nitrogen Compounds: The effect of modifying a medium by removing the nitrogen source was studied. The medium was Synthetic Medium C (described in Table I), the conditions were ambient, and the inoculation was made with 10^{10} Escherichia coli cells in 31 milliliters. The presence or absence of nitrogen in the medium apparently had no effect on the rate or extent of the potential changes because of the heavy inoculum. The results are shown in Figure 39.

Concentration of Yeast Extract: In one experiment, the concentration of yeast extract was varied from 0 to 0.5 percent in Synthetic Medium C (described in Table I). The microorganism used was Escherichia coli, and the inoculum concentration was 10^9 cells in 31 milliliters. The results are shown in Figure 40. The addition of yeast extract decreased the length of time required to produce a change of potential, from approximately 10 hours to 5 hours; the total potential change was apparently not affected. There was apparently no difference produced by the amount of yeast extract added, up to the 0.5 percent concentration level.

In another experiment, the concentration of yeast extract was varied from 0 to 0.1 percent in Synthetic Medium C (described in Table I). The inoculum was approximately 10^8 cells of

Escherichia coli in 31 milliliters, and the conditions were ambient. The addition of as little as 0.001 percent yeast extract decreased the time required to initiate a significant change of potential, but the time required to attain a total change of approximately 630 millivolts was essentially unchanged by the addition of yeast extract (see Figure 41).

4. Concentration of Inoculum

The effects of inoculum concentration upon the oxidation-reduction potential obtained with Escherichia coli were studied in various media.

The effects in nutrient broth were discussed in Section III. C. 1, where the inoculum sizes were 10^9 and 2×10^9 microorganisms per milliliter, and the results are shown in Figures 25 and 26.

In another experiment, the concentration of the nutrient broth was half of that described in Table I, and the concentrated inoculum was approximately 10^{10} cells in 31 milliliters. The results are shown in Figure 42, where it is again seen that there is a general trend for lower concentrations of organisms to require a longer period of time to produce a given change of potential.

In Synthetic Medium C with 1 percent glucose, the concentration of Escherichia coli was varied from 10^9 to 4×10^9 organisms per 31 milliliters. The results are shown in Figure 43; in this medium, as in nutrient broth, the highest concentration of organisms produced the most rapid change of potential. The time required to produce the total change of potential varied with the concentration of microorganisms, as tabulated below:

Concentration of Microorganisms in 31 milliliters	Time Required to Produce Maximum Potential Change (hours)
10^9	19
2×10^9	12
4×10^9	8

In another experiment, Escherichia coli was again inoculated into Synthetic Medium C at ambient conditions. The initial concentration of inoculum was 10^{10} cells in 31 milliliters, and the

culture was diluted four-fold at each dilution until it was one-two hundred fifty sixth (1/256) of its original concentration (4×10^7 cells in 31 milliliters). The total potential change was essentially unaffected by the dilution, but the time required to produce the total change of potential increased with decreasing concentration of microbes (see Figures 44 and 45).

A similar experiment was conducted with Escherichia coli inoculated into trypticase soy broth (described in Table I). The inocula were much smaller than those used in the above experiments, and ranged from 10^2 to 10^4 organisms per milliliter. The results are shown in Figure 46; even at as low a concentration of microorganisms as 10^2 per milliliter, there was a change of more than 600 millivolts in potential in 20 hours.

5. Temperature

Experiments were conducted to determine the effects of temperature on the oxidation-reduction potential produced by microorganisms in various media.

In one experiment, Escherichia coli at a concentration of approximately 2×10^{10} organisms in 31 milliliters was inoculated into Synthetic Medium C (described in Table I). The system was aerobic, and the incubation temperatures were 15, 25 and 35°C. The results are shown in Figure 47, where it is obvious that a higher temperature (35°C) produced more rapid changes of potential than lower temperatures, and that the time required to produce the potential change was roughly inversely proportional to the temperature. Further discussions of the effects of temperature on the oxidation-reduction potentials may be found in Section III.C.6, below.

6. Combined Effects of Modification of Media and Increased Temperature

Synthetic Medium C was modified as described in Table I (described as Modified Synthetic Medium C); further, yeast extract was added at a concentration of 0.1 gram per liter of medium.

The purpose of this modification of the medium was to decrease the length of time necessary for Escherichia coli to produce

a significant change of potential. The temperature of the modified medium was maintained at 37°C, in contrast to the ambient temperature (25°C.) of the Medium C system. The comparison between the two systems is presented below:

	Medium C	Modified Medium C
Temperature	25°C.	37°C.
Na_2HPO_4	6 g./L.	0.6 g./L.
Yeast extract	none	0.1 g./L.
Glucose	1 g./L.	10 g./L.

The data from these tests were averaged and are presented in Figure 48. The response time for a maximum 600 millivolts generated signal was 4 to 5-1/2 hours for the modified medium, while a potential change of only 200 millivolts was obtained with the unmodified medium after 20 hours.

7. Combined Effects of Temperature, Concentrations of Nutrient and Microorganisms, and Additions of Yeast Extract and Dextrose

An experiment was conducted in an attempt to optimize several variables simultaneously, and therefore decrease the amount of time required for the change of potential as much as possible. Previous experiments, in which only single variables were changed, had indicated that increased temperature (37°C), reduced concentration of nutrient (one-half full strength), increased number of microorganisms (10^8 cells per milliliter), and additions of yeast extract (0.1%) and dextrose (1%) decreased the amount of time required for the change of potential. The system chosen for this experiment was Escherichia coli in trypticase soy broth. Seven cells were run under the conditions described above, and the total potential change of 600 to 650 millivolts occurred within 40 to 45 minutes. Control cells were also run. The results of this experiment are presented in Table VII.

D. Preconditioned Media

A basic goal of the MARBAC research program was the production of significant (e.g., 100 millivolt) oxidation-reduction potential changes within 24 hours by small initial numbers of microbes (e.g., 100 microbes per milliliter of solution). A medium might be made suitable for rapid potential change by allowing microbes to grow in it

until the potential began to change significantly, but before a large (e.g., over 50 millivolt) potential change had occurred; the initial microbes would then be removed, and the bacteria-free medium used with a fresh inoculum. It was hoped that such a modified medium might change potential much sooner than the usual medium, with identical inocula.

Pilot test flasks were inoculated with 10^9 Escherichia coli bacilli per milliliter in Synthetic Medium C (described in Table I). The average time required for a change of potential was determined. The typical variation between duplicate flasks was less than 10 millivolts, and the average potential drops obtained at given intervals were as follows:

Time After Inoculation	Potential Change
2-1/2 hours	20 millivolts
3	30
3-1/2	50
4	90

Three hours was chosen as a suitable time to stop microbial metabolism in the medium. Test flasks were inoculated with the same concentration of Escherichia coli described above; at two and three-quarter hours after inoculation, all flasks were chilled to 0°C; by three and one-quarter hours all media had been centrifuged at 4°C and 12,000-g for 15 minutes. The medium was then filtered through 0.45 micron pore-size Millipore membrane. The average change in potential for all flasks was 50 ±5 millivolts up to the time the flasks were iced.

The medium thus modified was then inoculated with Escherichia coli at a lower concentration of microorganisms than was used in most of the other experiments in this program; i.e., at a concentration of 3×10^5 organisms per milliliter. The time required to obtain a change of potential with this medium was compared with that required for the usual Synthetic Medium C inoculated with the same concentration of Escherichia coli. There was a difference in the time of response of over two hours between the modified and unmodified media, as shown in Figure 49. The curves in Figure 49 represent the averages of four cells of the two inoculated media, and two controls for each uninoculated medium.

E. Correlations Between Oxidation-Reduction Potentials, Bacterial Counts, and pH

Four experiments were conducted, to determine the correlations between oxidation-reduction potentials and bacterial counts. In each case, the experimental conditions were ambient, the inoculations were made from cultures grown at 37°C. for 18 hours, and the medium was trypticase soy broth (described in Table I).

Escherichia coli was used in two experiments (see Figures 50 to 52, Bacillus subtilis in the third (see Figure 53), and Serratia marcescens in the fourth (see Figure 54). Particle counts were made by plating on agar, by means of optical density (turbidimetry), and by use of the Coulter counter; these techniques are described in Section II.B.2.

Plate and Coulter counts were averaged from four tests at each concentration. Typically, three dilutions of a serial set were tested, e.g., 10^5, 10^6, and 10^7 cells per milliliter. Plates were read which contained 30 to 300 colonies each.

In general, potential changes were proportional to bacterial counts. However, there appeared to be a tendency for the potential to change sooner than the bacterial count. This observation is in keeping with the established fact that bacteria are typically very active metabolically just prior to entering a logarithmic growth phase.

In another experiment, the standard trypticase soy broth medium (described in Table I) was modified by the addition of 1 percent glucose, and the temperature was varied from 27°C to 37°C, to determine the combined effects of these variables on the oxidation-reduction change, pH change, and cell multiplication rate. The bacterial species studied was Escherichia coli. The culture used as inoculum contained 2.41×10^9 cells per milliliter (Coulter count) and was 17-1/3 hours old. This was diluted to 2.41×10^7 cells per milliliter.

The addition of glucose to the trypticase soy broth, combined with an increase in temperature, decreased the time required for a change in the open-circuit potential, as shown in the following tabulation and in Figure 55:

Nutrient	Time Required for Potential Change (hrs)	
	Start of Significant Rate of Change	Completion of Significant Rate of Change
Trypticase Soy Broth	1-1/3	4
Trypticase Soy Broth + 1% added Glucose	1/3	2-1/6

The addition of glucose to the trypticase soy broth, combined with the increase in temperature, also decreased the total change in potential (from 775 millivolts to 625 millivolts). These effects are also shown in Figure 55.

The combined effects of the addition of glucose and increase in temperature also resulted in an increased rate of multiplication of the microorganisms (as shown in Figure 56), as well as an increased rate of change of the pH (see Figure 57).

Bacterial counts correlated closely with the nature and extent of oxidation-reduction (redox) potential changes in trypticase soy broth, produced by Escherichia coli at 27°C and 37°C. (See Figure 58). Based on potential readings taken every 10 minutes, there appeared to be two distinct portions of potential change in the given medium, which are discernible in the curve at 27°. The initial portion covered a 200 to 300 millivolt range (starting at about +300 millivolts, standard hydrogen-platinum scale) and involved 100 millivolt changes in 20 minutes or longer. The second potential drop covered a 400 to 500 millivolt range (starting at about +100 millivolts), and involved 100 millivolt changes in 10 minutes or less. Bacterial electronic (Coulter) counts were approximately 10^7 organisms per milliliter at the beginning of the first large potential change, and approximately 10^8 organisms per milliliter at the beginning of the second large potential change. The distinct portions of the overall redox curve for trypticase soy broth may provide clues to the biochemical or other factors involved in the potential change of this medium.

Bacterial counts correlated consistently with redox changes in modified trypticase soy broth (glucose 1.25 percent and yeast extract 0.01 percent, Table I) when compared to the unmodified medium. The counts for different points of potential change in modified broth are shown in Figure 59. At the point of greatest total potential change, the counts for three different trypticase soy broth media (varied composition and temperature) were 210, 216, and 223 X 10^6 microbes per milliliter, respectively. The bacterial count in trypticase soy broth finally reached 8 X 10^8 organisms per milliliter, but by the time an approximate count of 4 X 10^8 was present, the maximum potential change had occurred.

Glucose was added (to a final concentration of 1.25 percent) to trypticase soy broth to provide large-scale pH changes, which were studied with a Beckman Research pH Meter (Model 101900) with a relative accuracy of 0.001 pH units. Unmodified trypticase soy broth (0.25% glucose) changed 0.63 pH units over a 6-1/2 hour period, while broth with 1.25 percent glucose changed 1.47 pH units (Figure 60). Converted to a potential scale, the pH changes were 63 millivolts for normal broth and 147 millivolts for glucose broth. At the same time, redox potential changes were 700 millivolts for normal broth and 500 millivolts for 1.25 percent glucose broth. The increase of acidity in trypticase soy broth produces a potential which is opposite the demonstrated direction of redox potential change. Thus, in trypticase soy broth, in order to enhance redox change it would be desirable that there be no change toward acidity (i.e., decrease of pH).

F. Miniaturization of Test Cells

Initial studies using small volumes (0.05 milliliter) of test solution produced a smaller change of potential, and required a longer time than the larger volumes (31 milliliters) usually used in the experiments of this program. In the smaller volume, the time required to reach the maximum change of potential was more than two hours longer than the six hours required by the larger volume. Medium and organism concentrations were identical for both volumes; a given mixture of microbe and medium was first prepared in a flask and then transferred to large and small test setups.

In one test, nitrogen gas was used to overlay the 0.05 milliliter drop, in order to prevent oxygen absorption from the atmosphere by the relatively large ratio of surface area to volume of the small drop. No effect of the nitrogen overlay was noted with total potential change. The results of this experiment are shown in Figure 61. The system chosen for this experiment was one about which a considerable amount of information had been obtained in the past; i.e., Escherichia coli in trypticase soy broth, grown at 37°C. The initial concentration of microorganisms used in the experiment was approximately 10^7 cells per milliliter.

Another experiment was run, in which the volume of bacteria and nutrient was approximately 0.4 milliliter. The conditions of the experiment were the same as those described above.

The results of this experiment, judged by the variation of open-circuit potential with time, showed excellent agreement with the results obtained with the larger volume of media and microorganisms. (See Figure 62).

Another experiment was conducted, to determine whether the rapid increase in potential shown in Figure 62, beginning approximately seven hours after inoculation,could have been caused by the entrance of oxygen (from air) into the cell. The effect of oxygen at the surface of the liquid had been minimized when experiments were initiated with the small volume of liquid (0.4 milliliter) by simultaneously reducing the area of liquid exposed to the oxygen; that is, different configurations of test cells were used in the large liquid volume and in the small liquid volume experiments. The test cells are compared below:

Liquid Volume (ml.)	Surface Area (sq. cm.)	Liquid Depth (cm.)	Description
0.4	0.50	0.8	Cylinder
31.0	14.0 (Approx.)	2.8 at deepest point	3 Cylindrical necks superimposed on a sphere

Although the ratios of surface area to volume were not the same for the two types of cells, at least the surface area was reduced for the small volume cell in comparison to the large volume test cell.

The possible effect of oxygen was further minimized in this experiment by blanketing the liquid with nitrogen. Escherichia coli again was inoculated into trypticase soy broth, and the surface was blanketed with nitrogen. It seemed that excluding oxygen from the cell was successful in stabilizing the potential, although it did not appreciably affect the total change of potential, which was approximately 800 millivolts in both cases. These results are shown in Figure 63.

A comparison of the bottom curve of Figure 63 with Figure 61 indicates that experimental cells using 0.4 milliliter of solution and microorganisms can be operated to produce essentially the same changes in potential as the larger (31 milliliter volume) cells.

IV. CONCLUSIONS

Instrumentation used in studying the changes in oxidation-reduction potentials produced in various nutrient-microorganism systems has included both a 50-channel and 100-channel automatic recording, digital voltmeter. The voltmeters had input impedances of 10^7 and 10^9 ohms, respectively.

The reproducivility of potential readings was acceptable, and was within a 20 millivolt range under favorable conditions. It was found that the experimental test cells could be maintained in a sterile condition for extended periods of time.

Bacterial counts were made with a Coulter Counter, which is faster and more accurate than the usual methods employed in counting bacterial particles, such as agar plate counts and turbidimetric measurements. Agar plate counts were made in determining numbers of viable microorganisms.

The electrode pairs were platinum and saturated calomel. Electrode surface contamination was minimized by employing Luggin capillaries with the saturated calomel reference electrodes, and by protecting surfaces of the platinum wire electrodes.

It was established that changes of potential occurred only in media in which microbes could grow. Escherichia coli was used as the primary test organism in this program, because it has been the subject of more extensive metabolic research than any other microorganism.

The effects of various growth media and growth factors upon the variation of the oxidation-reduction potential with time were examined. Under favorable conditions (i.e., a heavy inoculum concentration in a suitable growth medium), potential changes as high as 600 millivolts were obtained in as little as 3-1/2 hours with this organism.

Other microorganisms were studied in this investigation, including Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus subtilis, Alcaligenes faecalis, Erwinia caratovora, Aerobacter aerogenes, Streptococcus faecalis, and Serratia marcescens. These microorganisms were inoculated into various of eleven standard or modified media. The extent and rate of change of potential in these systems varied, but in all cases the presence of bacteria could be detected (in a suitable medium) by a change of potential.

Twelve desert soil samples, such as those that might be found on Mars, were obtained from Dr. Roy Cameron, of the Jet Propulsion Laboratory. These samples represented a reasonably broad spectrum of soils. Various of these soils were inoculated into trypticase soy broth, and potential changes were produced by the indigenous microorganisms in each case. Changes in potential as high as 690 millivolts were obtained.

Garden soil was added to enrichment media, to select various microorganisms; changes in potential indicated growth of some of the microorganisms.

The effects of variables upon the extent and rate of change of potential were studied. The variables included the concentration of the medium, aerobic and anaerobic conditions, modifications of the media, concentrations of the inocula, temperature, and combinations of the above variables. In general, it was found that dilution of the medium resulted in a smaller change of potential and more time to obtain a given change of potential.

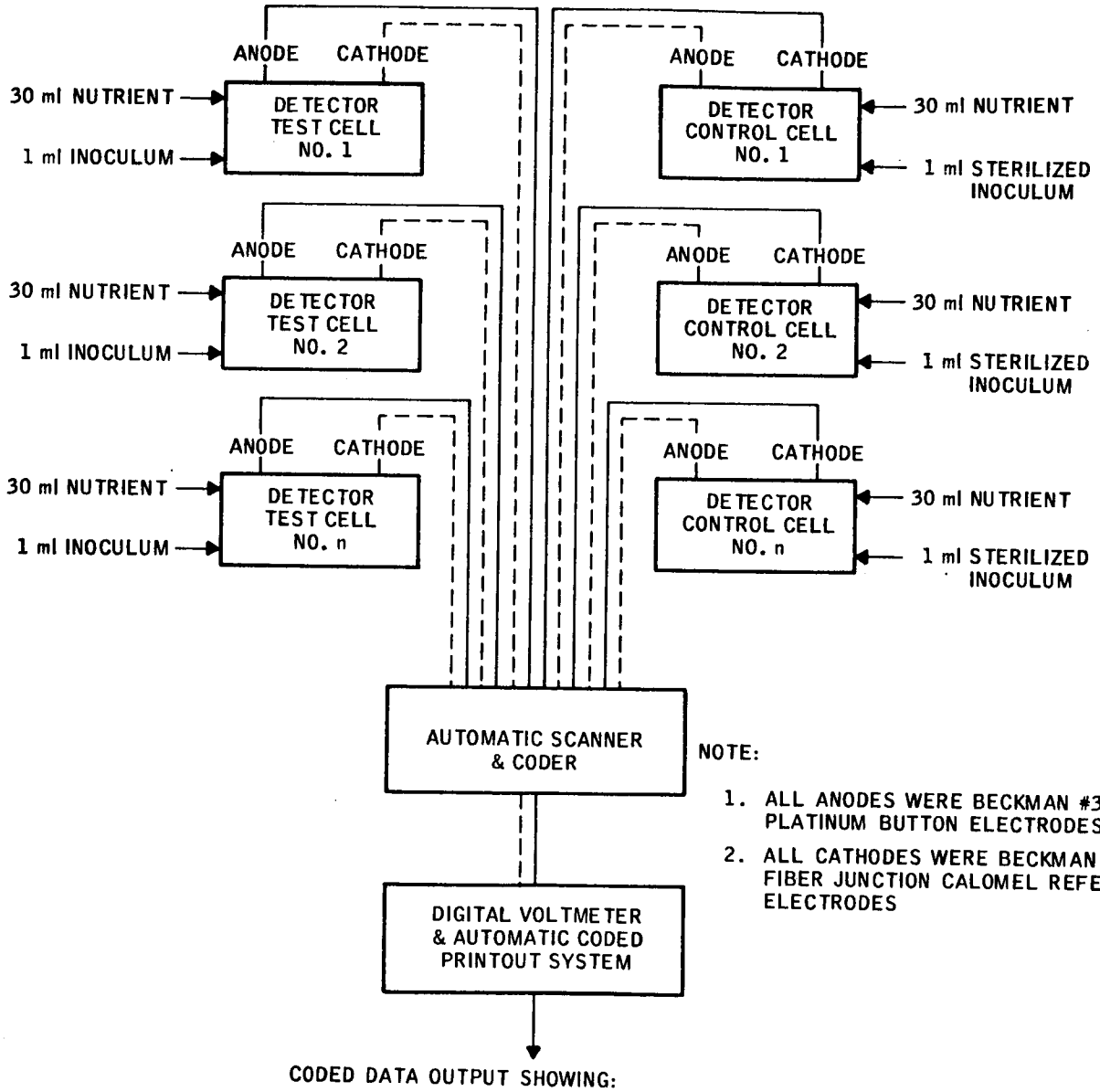
There was little effect of anaerobic or aerobic conditions if the inoculum concentration was high. Modifications of the media were effective in changing the potential; concentrations of 1 percent glucose, 0 percent thioglycollate, 0 percent nitrate, and less than 0.1 percent of Na_2HPO_4 were most effective in producing potential changes. The addition of small amounts of yeast extract resulted in more rapid changes of potential. Lower concentrations of inocula required longer periods of time to produce changes in potential. Higher temperatures (35°C) produced more rapid changes of potential. The effects of some combined variables were also studied, and it was found that increasing the temperature (to 37°C), adding yeast extract (0.1 gram per liter), and reducing the concentration of Na_2HPO_4 (to 0.6 gram per liter) in Synthetic Medium C resulted in far more rapid changes in potential.

An attempt was made to precondition the medium by allowing microorganisms to grow in it prior to test. This technique resulted in a more rapid response as exhibited by a change of potential.

Correlations were developed between oxidation-reduction potentials, bacterial counts, and pH. A change of potential was detected sooner than a change in bacterial count, indicating active metabolism prior to growth.

Studies of the effects of miniaturizing the test cells indicated that experimental cells containing 0.4 milliliter of nutrient and microorganisms could be operated to produce essentially the same changes in potential as larger cells containing 31 milliliters of the nutrient-microorganism mixture.

TYPICAL LABORATORY TEST SETUP AND INSTRUMENTATION



NOTE:

1. ALL ANODES WERE BECKMAN #39281 PLATINUM BUTTON ELECTRODES
2. ALL CATHODES WERE BECKMAN #39270 FIBER JUNCTION CALOMEL REFERENCE ELECTRODES

CODED DATA OUTPUT SHOWING:

1. CHANNEL NUMBER
2. VOLTAGE (INCLUDING DECIMAL POINT & SIGN)
3. TIME

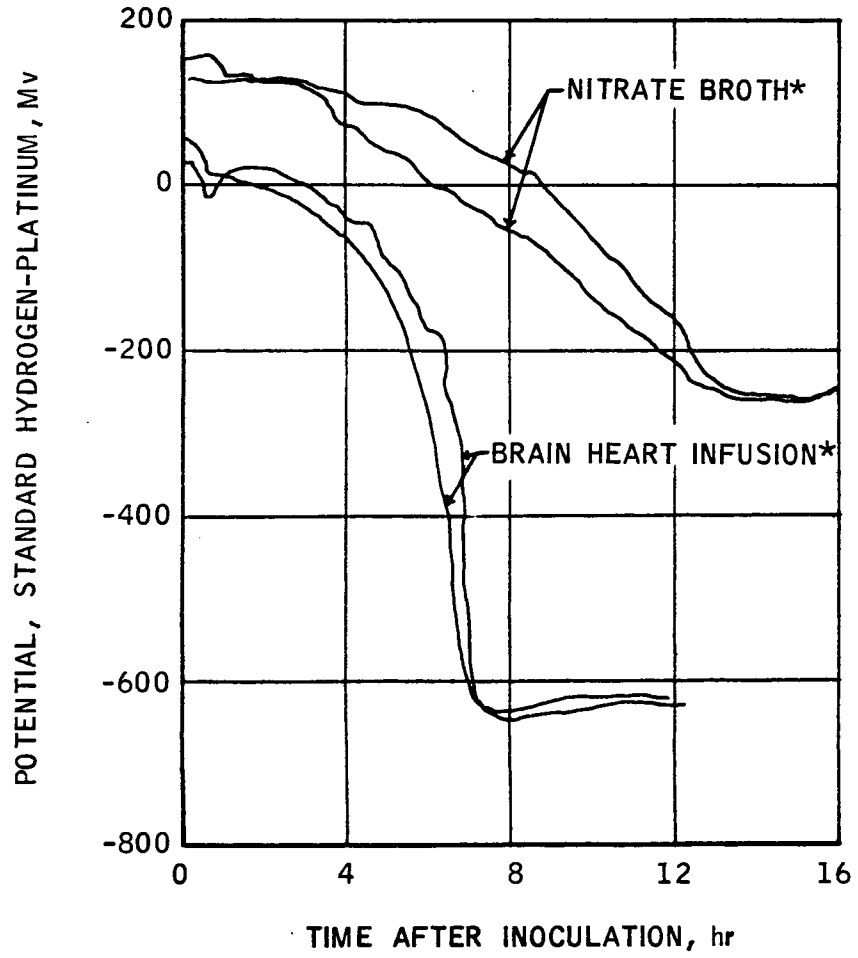
Figure 1

MARBAC DIGITAL VOLTMETER
AND DATA ACQUISITION SYSTEM

Neg. 6161-1

Figure 2

REPRODUCIBILITY OF POTENTIAL CHANGES WITH TIME IN IDENTICAL TESTS



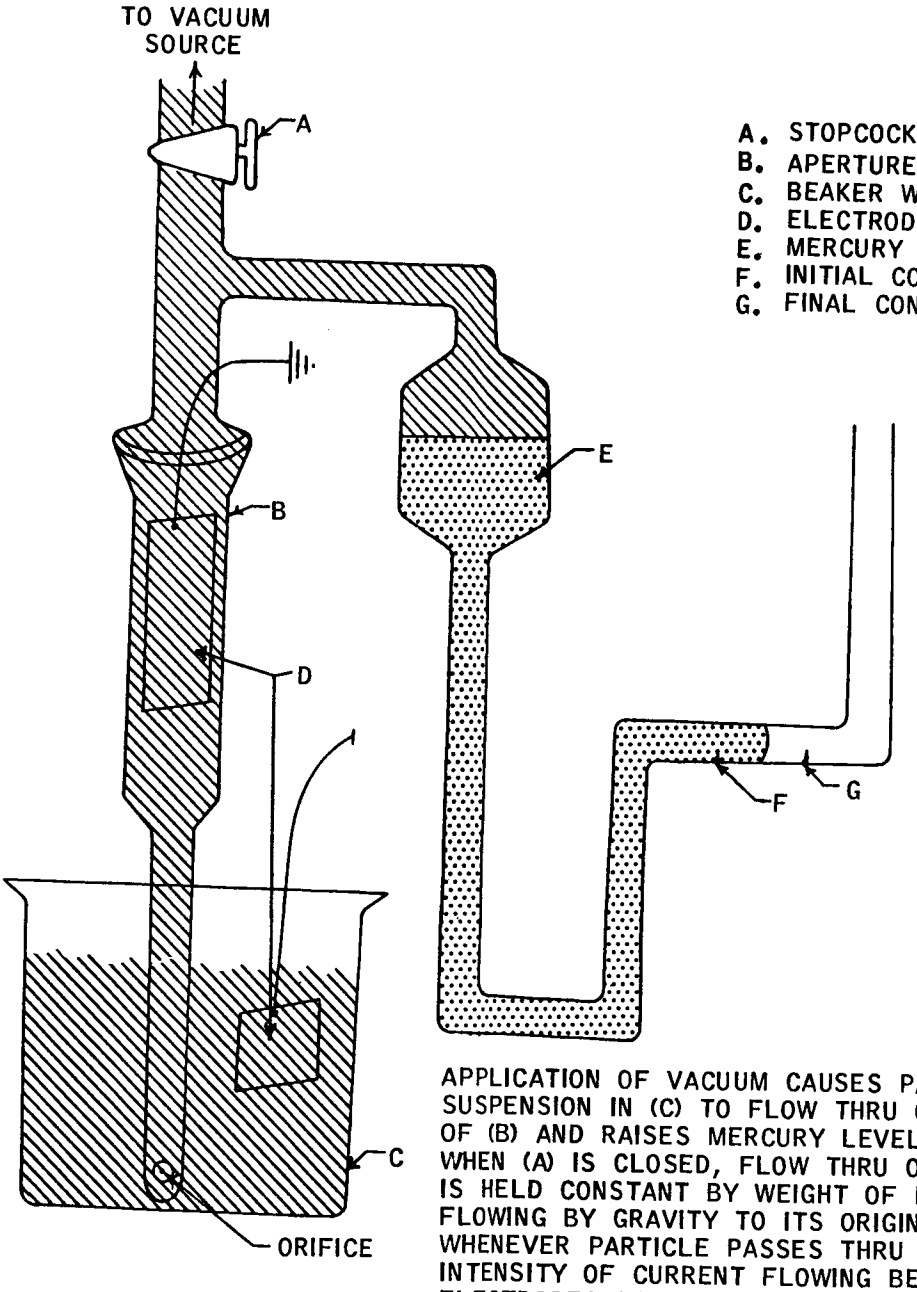
(SPECIFIC DETAILS OF THE EXPERIMENTS FROM WHICH THESE CURVES WERE TAKEN ARE OMITTED, BECAUSE ONLY REPRODUCIBILITY IS BEING ILLUSTRATED.)

*DESCRIBED IN TABLE I

Figure 3

COULTER COUNTER

ELECTROLYTIC RESISTANCE MEASUREMENT



A. STOPCOCK
B. APERTURE TUBE
C. BEAKER WITH SAMPLE
D. ELECTRODES
E. MERCURY
F. INITIAL CONTACT
G. FINAL CONTACT

APPLICATION OF VACUUM CAUSES PARTICLE SUSPENSION IN (C) TO FLOW THRU ORIFICE OF (B) AND RAISES MERCURY LEVEL (E). WHEN (A) IS CLOSED, FLOW THRU ORIFICE IS HELD CONSTANT BY WEIGHT OF MERCURY FLOWING BY GRAVITY TO ITS ORIGINAL LEVEL. WHENEVER PARTICLE PASSES THRU ORIFICE, INTENSITY OF CURRENT FLOWING BETWEEN ELECTRODES (D) IS CHANGED. CHANGES ARE TRANSLATED INTO ELECTRONIC PULSES VISIBLE ON DECADE BANK. CURRENT CONTROL PERMITS ADJUSTMENT OF PULSE HEIGHT TO SIZE RANGE UNDER STUDY. PULSES ARE COUNTED ELECTRONICALLY FROM MOMENT MERCURY REACHES FIRST CONTACT (F) TO MOMENT IT REACHES FINAL CONTACT (G).

Figure 4

POLARIZATION STUDY

COULTER COUNTER

SETTING: APERTURE CURRENT 0.354
GAIN 8
RESET LEFT
100 μ APERTURE
- - - - - - POTENTIAL
——— CURRENT

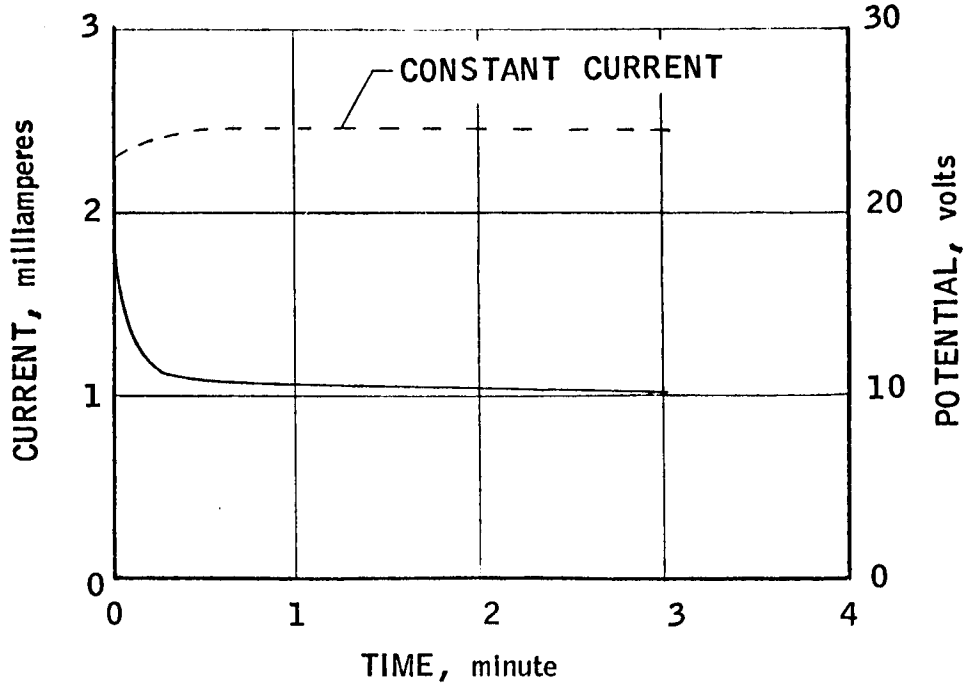
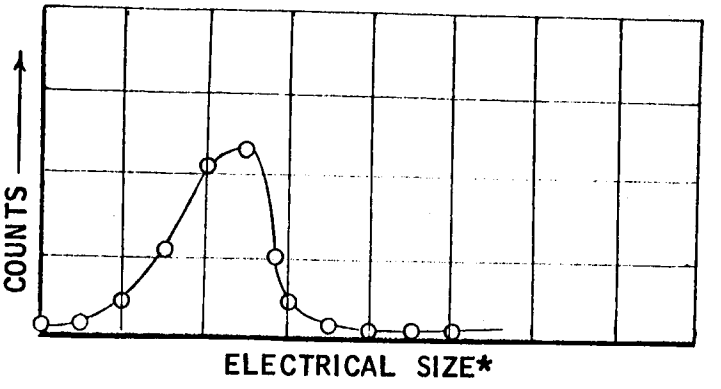


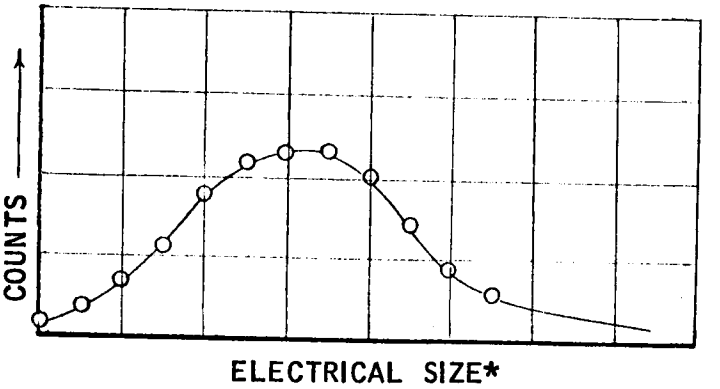
Figure 5

COULTER COUNTS AS RELATED TO CURRENT CONSTANCY

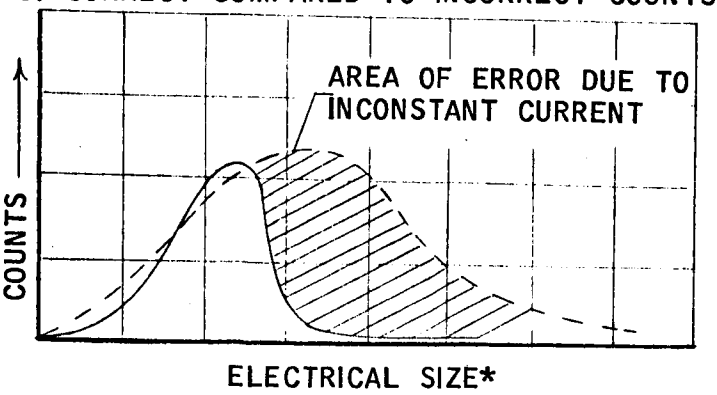
A. CORRECT COUNTS; CURRENT CONSTANT



B. INCORRECT COUNTS; INCONSTANT CURRENT



C. CORRECT COMPARED TO INCORRECT COUNTS

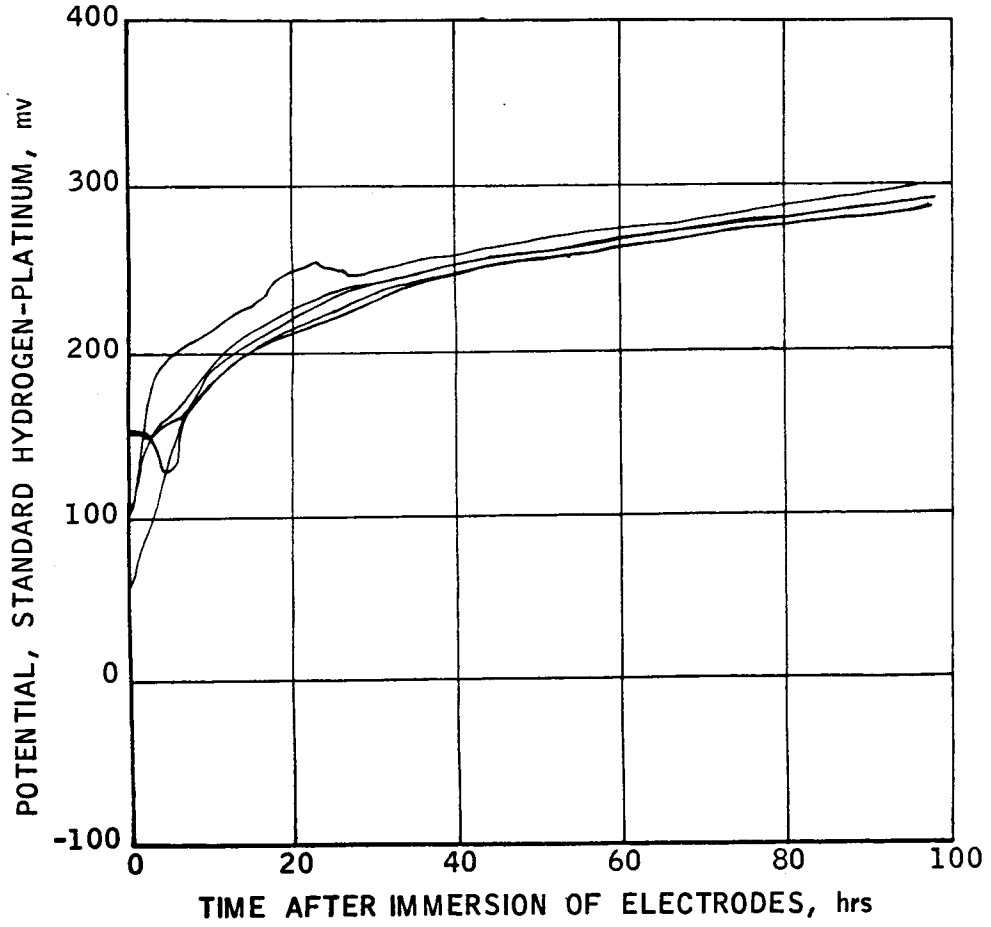


*ELECTRICAL SIZE IS DERIVED FROM THE CHANGE OF RESISTANCE PRODUCED BY PARTICLES FLOWING THROUGH THE APERTURE

Figure 6

REPRODUCIBILITY OF POTENTIALS PRODUCED IN TRYPTICASE SOY BROTH*

INOCULUM: 1 gram SOIL IN 10 ml STERILE, DEIONIZED WATER, SHAKEN 10 minutes
INOCULUM SIZE: 1 ml ABOVE SUSPENSION (AFTER SETTING) IN 30 ml OF MEDIA* (APPROXIMATELY)



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

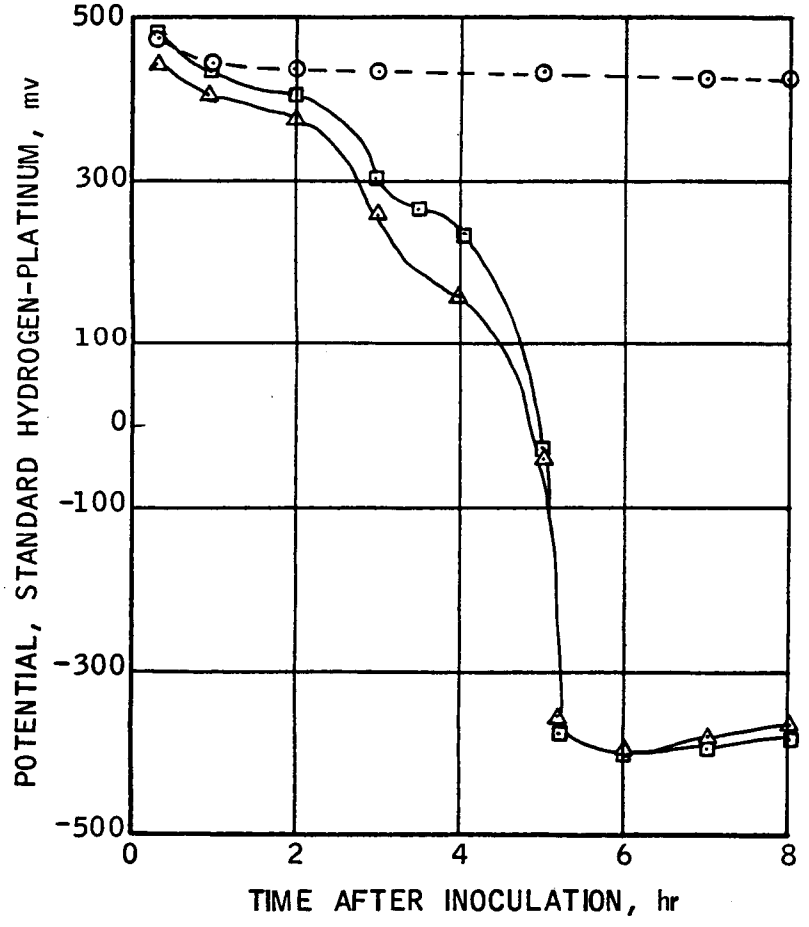
ATMOSPHERE = AMBIENT, NO AGITATION

ASEPTIC

* DESCRIBED IN TABLE I

Figure 7

COMPARISON OF CONVENTIONAL ELECTRODES WITH OTHERS WHICH CAN BE AUTOCLAVED



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT, NO AGITATION AFTER INOCULATION

ASEPTIC

MEDIUM = TRYPTICASE SOY BROTH*

— — — UNINOCULATED

——————— INOCULATED

○ = AVERAGE OF 6 UNINOCULATED CONTROLS

□ = AVERAGE OF 2 TESTS USING LUGGIN CAPPILLARIES FILLED WITH SALINE

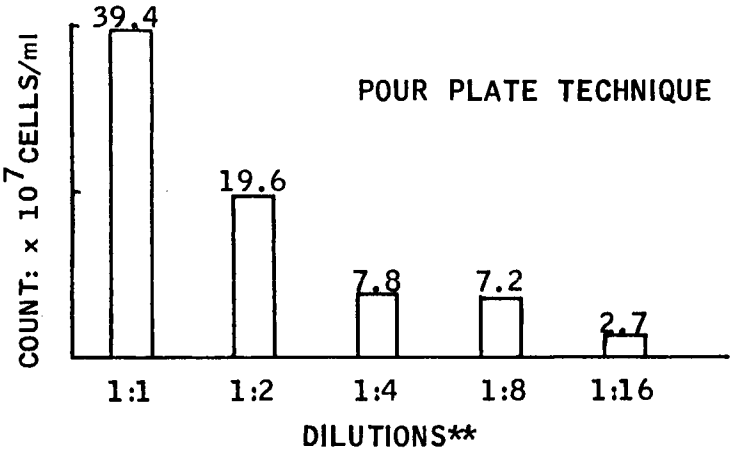
△ = AVERAGE OF 2 TESTS USING REGULAR CALOMEL ELECTRODES AND 2 TESTS USING LUGGIN CAPILLARIES FILLED WITH SATURATED KCl

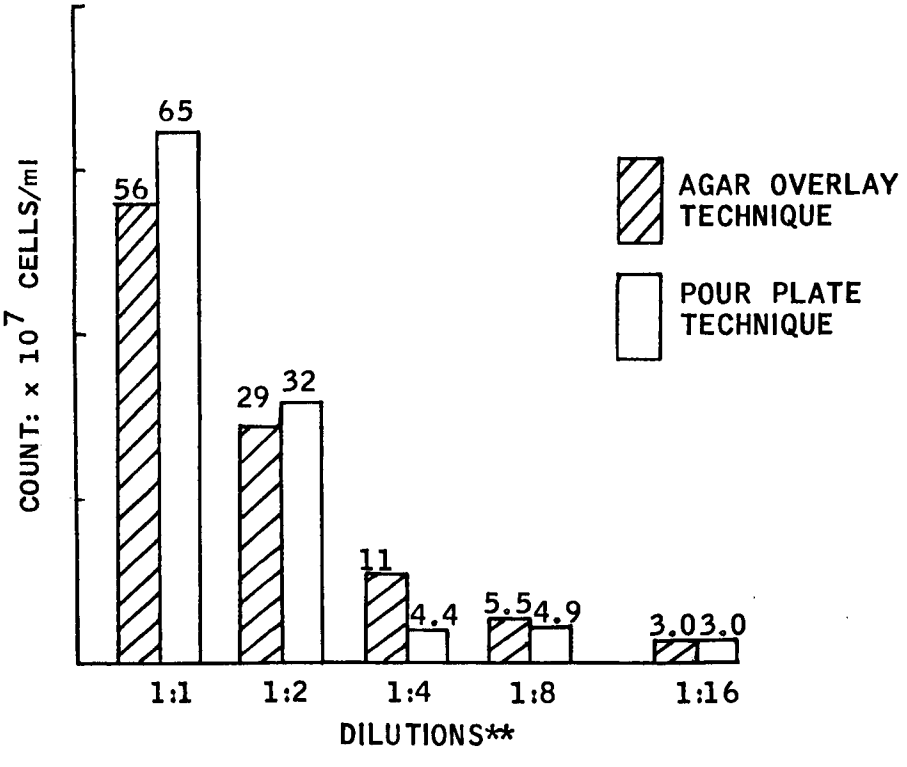
* DESCRIBED IN TABLE I

Figure 8

PLATE COUNTS OF E. COLI SUSPENSION IN 0.85% SALINE

PLATE MEDIUM*: TRYPTICASE SOY AGAR
ORGANISMS*: APPROX. 20 HOURS OLD, WASHED ONCE



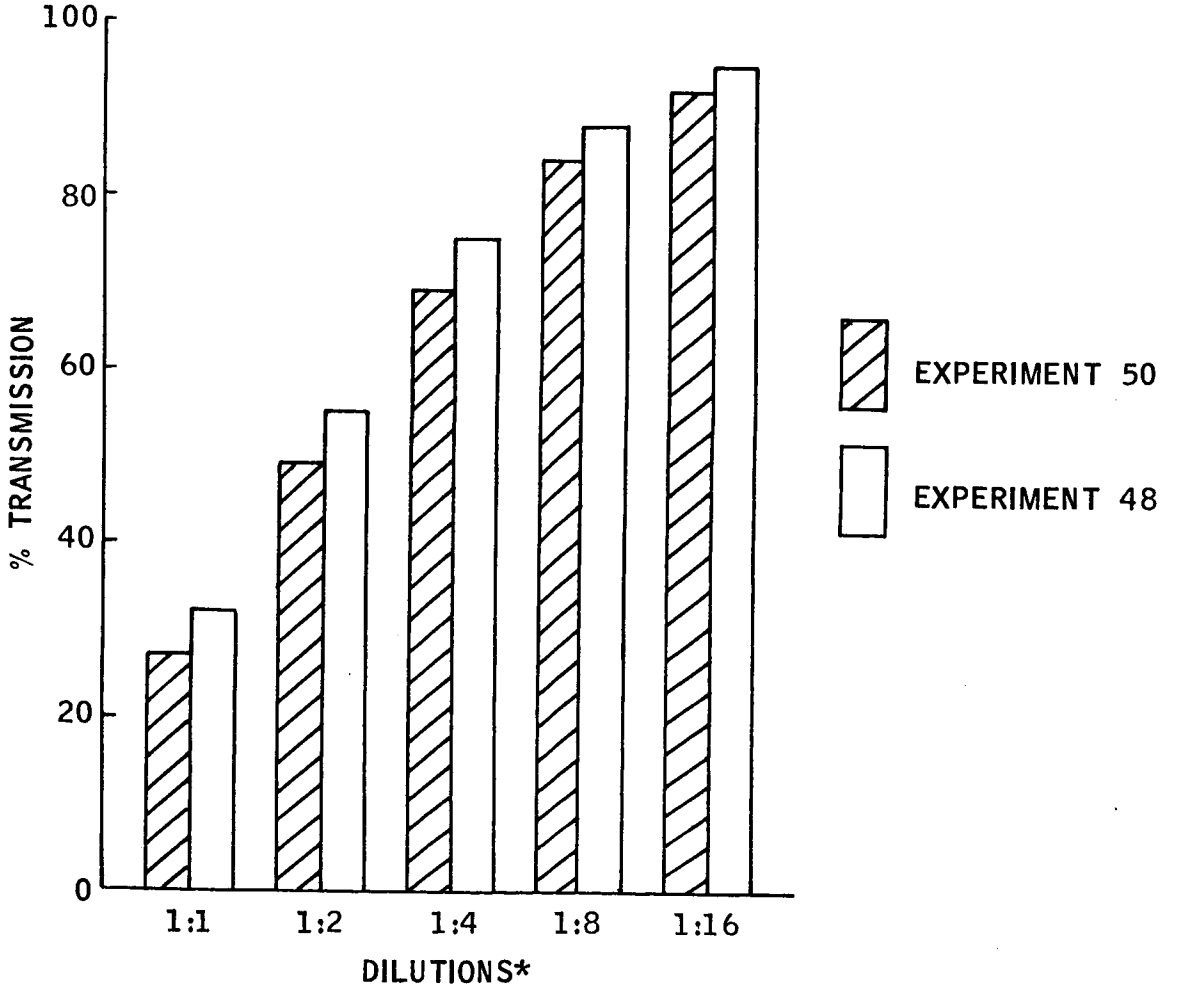


*APPLIES TO BOTH GRAPHS
**1:1 REPRESENTS SEDIMENTED CELLS RESUSPENDED IN SALINE OF EQUAL VOLUME TO THE ORIGINAL GROWTH VOLUME

Figure 9

TURBIDIMETRIC MEASUREMENT OF *E. COLI* SUSPENSION IN 0.85% SALINE

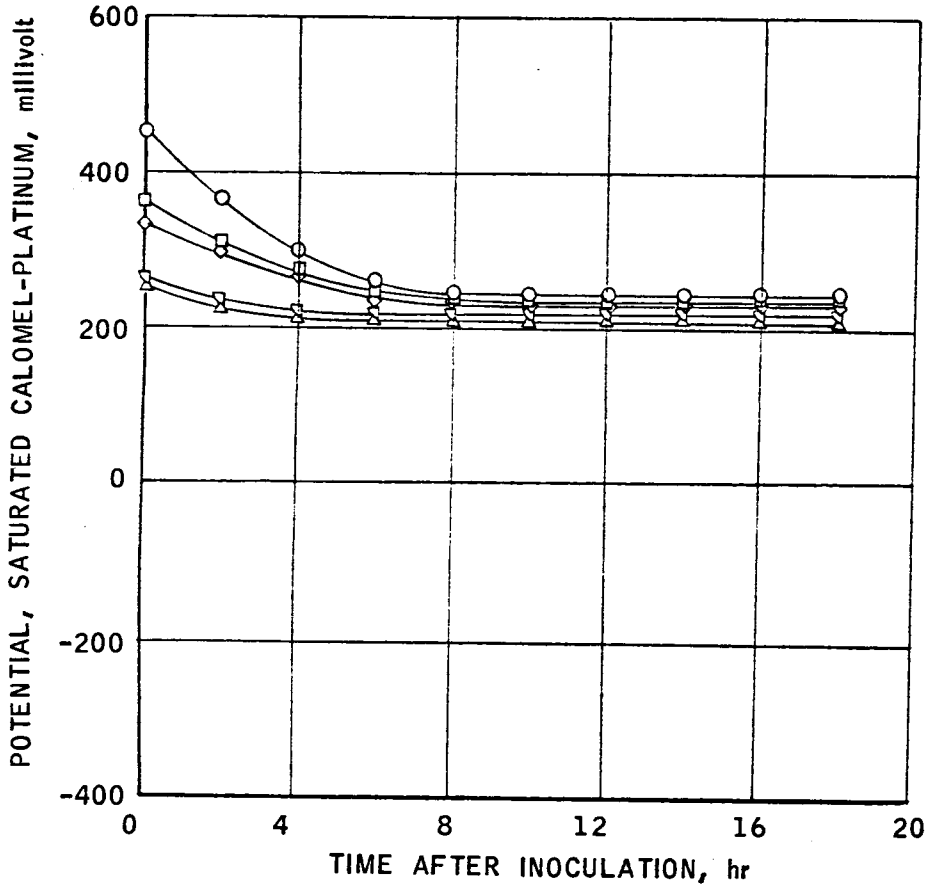
WAVELENGTH: 540 millimicrons
INSTRUMENT: COLEMAN UNIVERSAL SPECTROPHOTOMETER



*1:1 REPRESENTS SEDIMENTED CELLS RESUSPENDED IN SALINE OF EQUAL VOLUME TO THE ORIGINAL GROWTH VOLUME

Figure 10

EFFECT OF VARIOUS CONTROL SYSTEMS ON POTENTIAL CHANGE PRODUCED BY E. COLI



TEMPERATURE = 24 - 25° C
ATMOSPHERE = AMBIENT, NO AGITATION AFTER INOCULATION

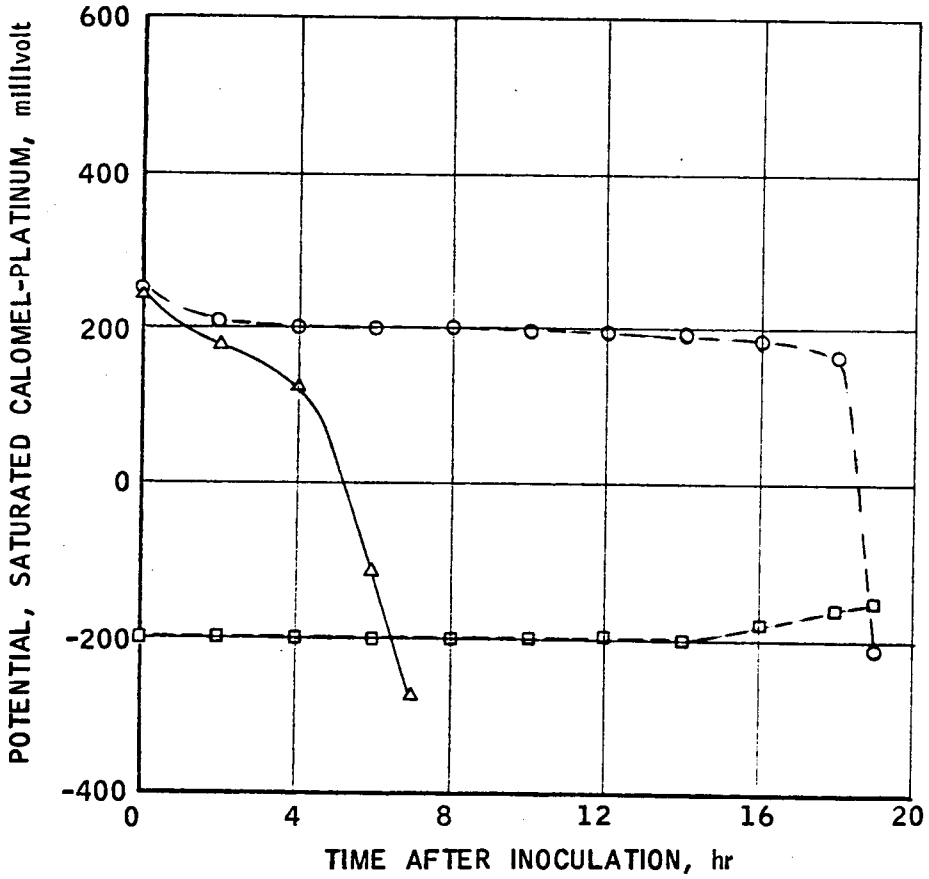
——— INOCULATED

○ = CONCENTRATED ORGANISM, 0.9% SALINE SOLUTION
□ = LIGHT INOCULUM, 0.9% SALINE SOLUTION
◇ = 0.9% SALINE SOLUTION
▽ = SYNTHETIC OR COMPLEX AEROBIC GROWTH MEDIUMS*, STERILE
△ = INOCULATED MEDIUM WITHOUT GROWTH

*DESCRIBED IN TABLE I

Figure 11

EFFECT OF VARIOUS CONTROL SYSTEMS ON POTENTIAL CHANGE PRODUCED BY E. COLI



TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT, NO AGITATION AFTER INOCULATION

– – – – UNINOCULATED
——— INOCULATED

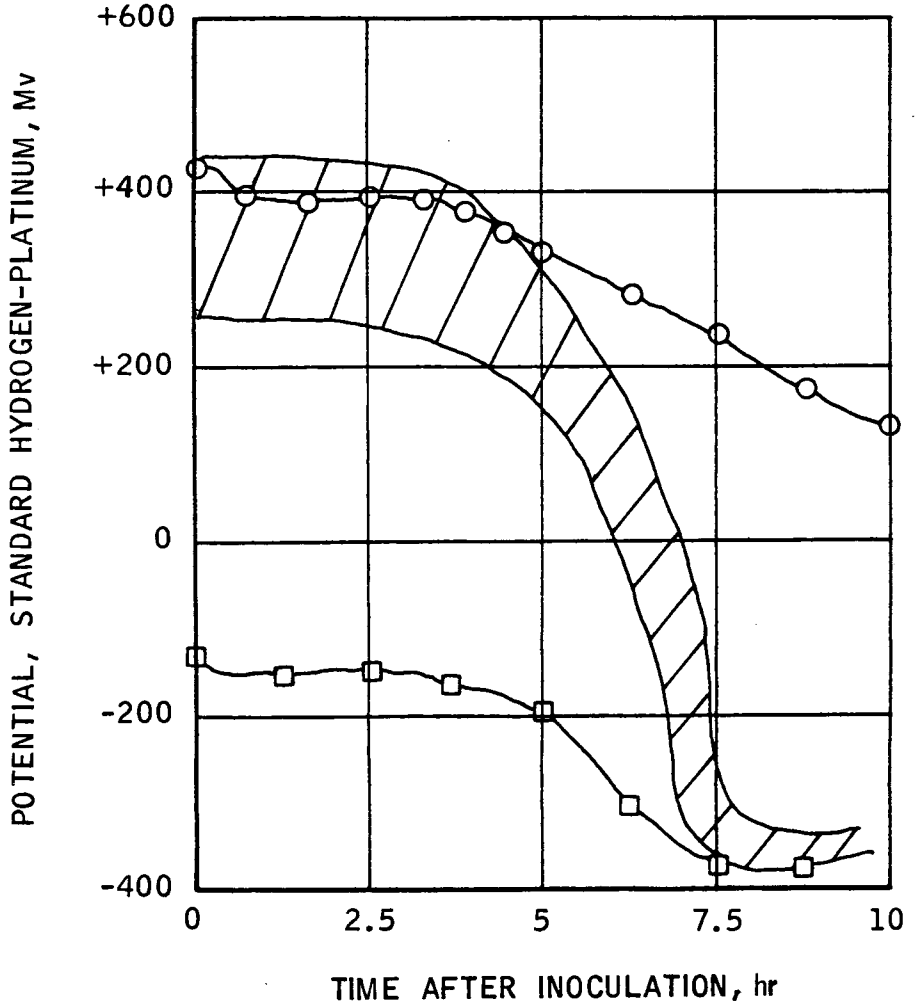
△ = INOCULATED MEDIUM*
□ = ANAEROBIC GROWTH MEDIUM*, STERILE
○ = GROWTH MEDIUM*, CONTAMINATED

*DESCRIBED IN TABLE I

Figure 12

CHANGE OF POTENTIAL PRODUCED BY *E. COLI* IN VARIOUS MEDIA*

INOCULA: 8 HOUR CULTURE, GROWN AT 37°C IN TRYPTICASE SOY BROTH
INOCULA SIZE: APPROXIMATELY 3 X 10^6 ORGANISMS/MILLILITER



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

TEST MEDIA:

○ = NITRATE BROTH

□ = THIOGLYCOLLATE BROTH

▨ = BRAIN HEART INFUSION, SYNTHETIC MEDIUM C, TRYPTICASE SOY BROTH, NUTRIENT BROTH

*DESCRIBED IN TABLE I

Figure 13

COMPARISON OF POTENTIAL DROP PRODUCED BY E. COLI IN DIFFERENT MEDIA

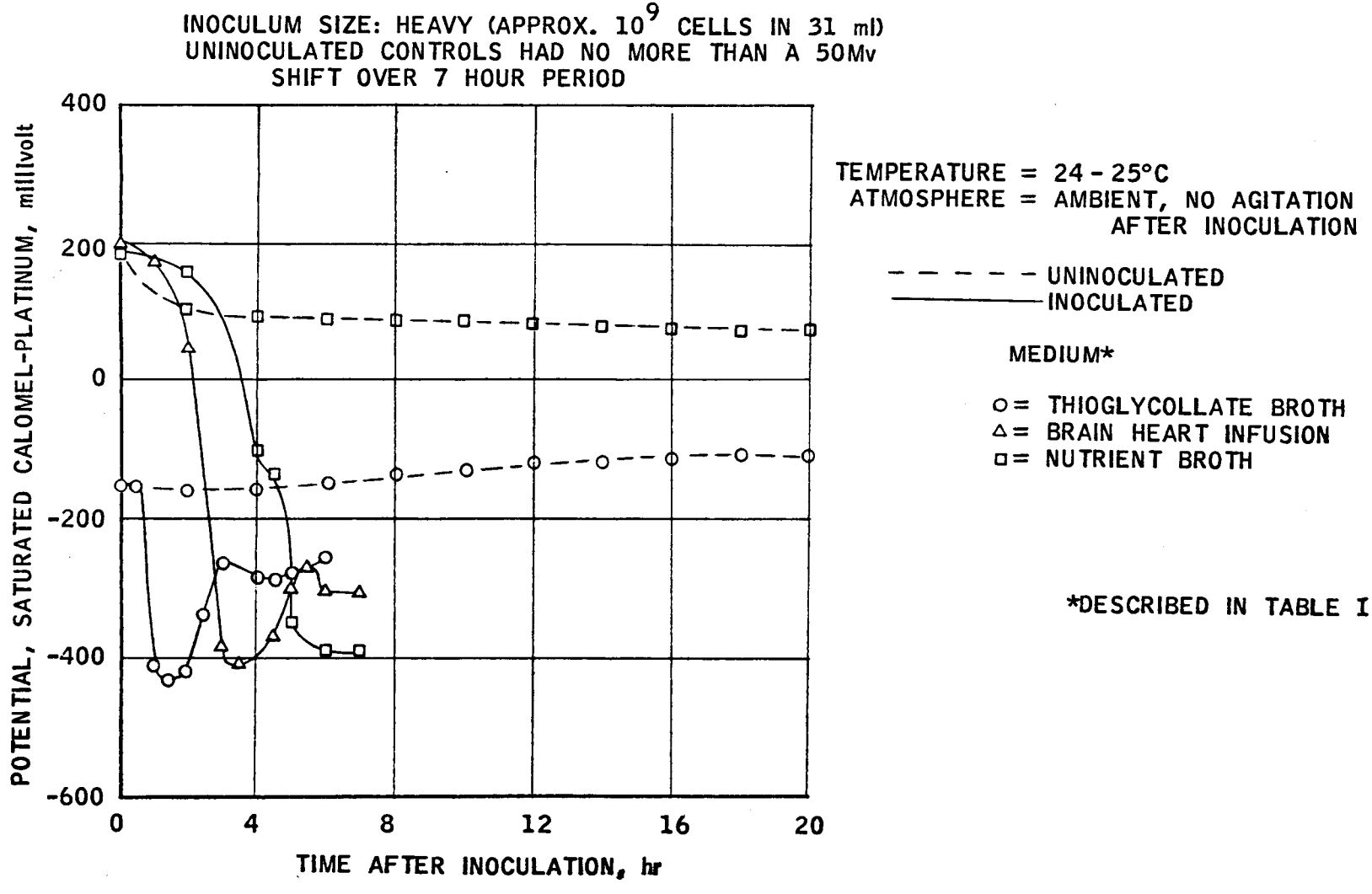


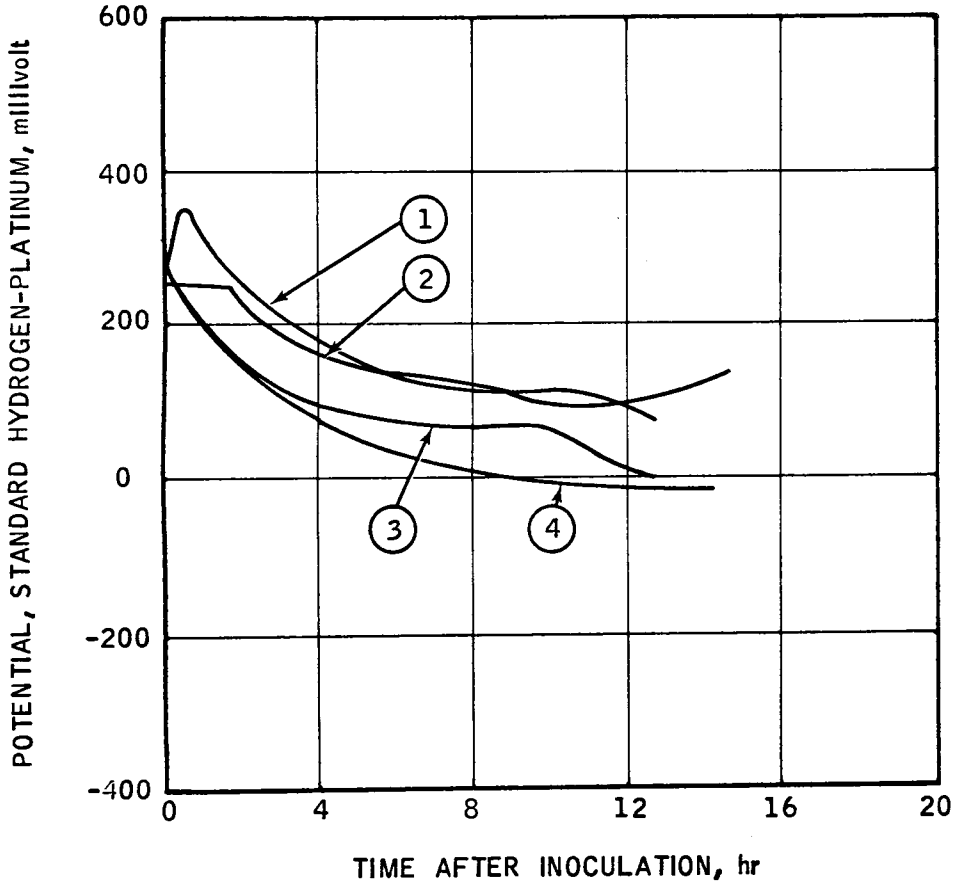
Figure 14

POTENTIAL CHANGES PRODUCED IN COMPLEX MEDIA BY PSEUDOMONAS AND BACILLUS

EXPERIMENT NO. 36

INOCULA: PSEUDOMONAS AND BACILLUS, 48 HR CULTURES, GROWN AT 24°C IN BRAIN HEART INFUSION BROTH

INOCULA SIZES: APPROXIMATELY 10^8 ORGANISMS IN 31 ML



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

MEDIUM AND ORGANISM

(1) = BRAIN HEART INFUSION* PSEUDOMONAS
(2) = NUTRIENT BROTH* - PSEUDOMONAS
(3) = NUTRIENT BROTH - BACILLUS
(4) = BRAIN HEART INFUSION - BACILLUS

*DESCRIBED IN TABLE I

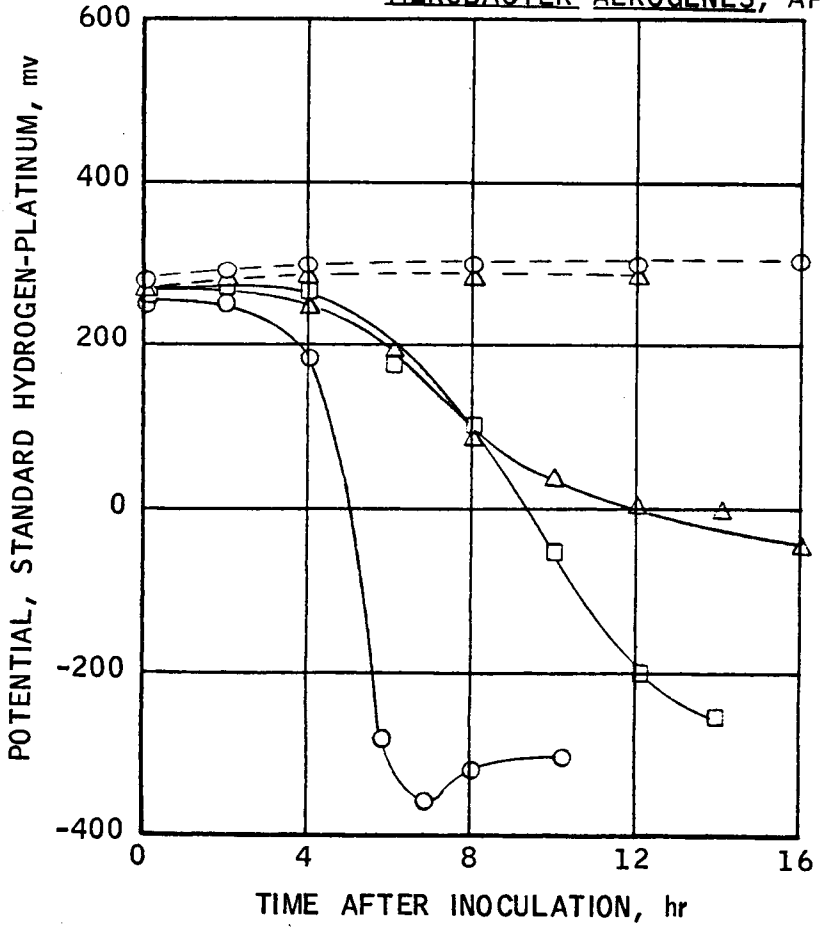
Figure 15

CHANGES OF POTENTIAL PRODUCED BY A. FAECALIS, E. CARATOVORA, AND A. AEROGENES IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 57

INOCULA: ALL 24 hr CULTURES GROWN AT 37°C

INOCULA SIZES: ALCALIGENES FAECALIS, APPROX. 10^8 ORGANISMS IN 31 ml
ERWINIA CARATOVORA, APPROX. 10^8 ORGANISMS IN 31 ml
AEROBACTER AEROGENES, APPROX. 10^9 ORGANISMS IN 31 ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION

ASEPTIC

- - - - - - UNINOCULATED

——— INOCULATED

TESTS:

△ = ALCALIGENES FAECALIS

□ = ERWINIA CARATOVORA

○ = AEROBACTER AEROGENES

*DESCRIBED IN TABLE I

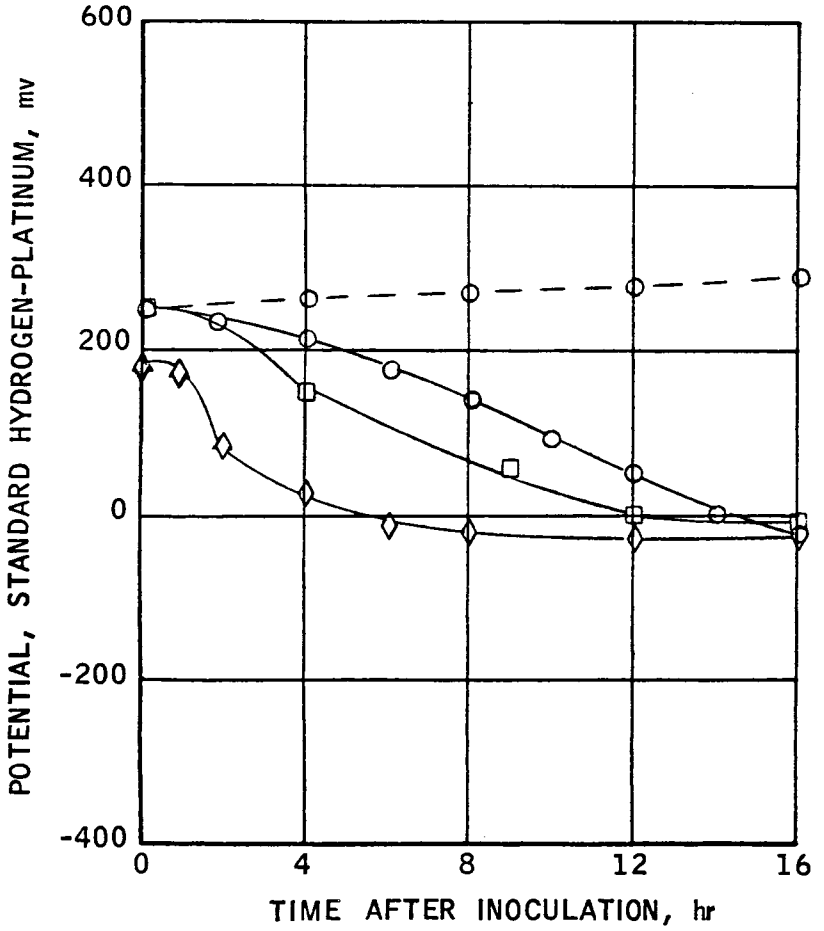
Figure 16

CHANGES OF POTENTIAL PRODUCED BY B. CEREUS, B. SUBTILIS, AND P. FLUORESCENS IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 58

INOCULA: ALL 23 hr CULTURES GROWN AT 37°C

INOCULA SIZES: ALL INOCULA APPROX. 10^8 ORGANISMS IN 31 ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION

ASEPTIC

- - - - - - UNINOCULATED

———— INOCULATED

TESTS:

○ = BACILLUS CEREUS

□ = BACILLUS SUBTILIS

◇ = PSEUDOMONAS FLUORESCENS

*DESCRIBED IN TABLE I

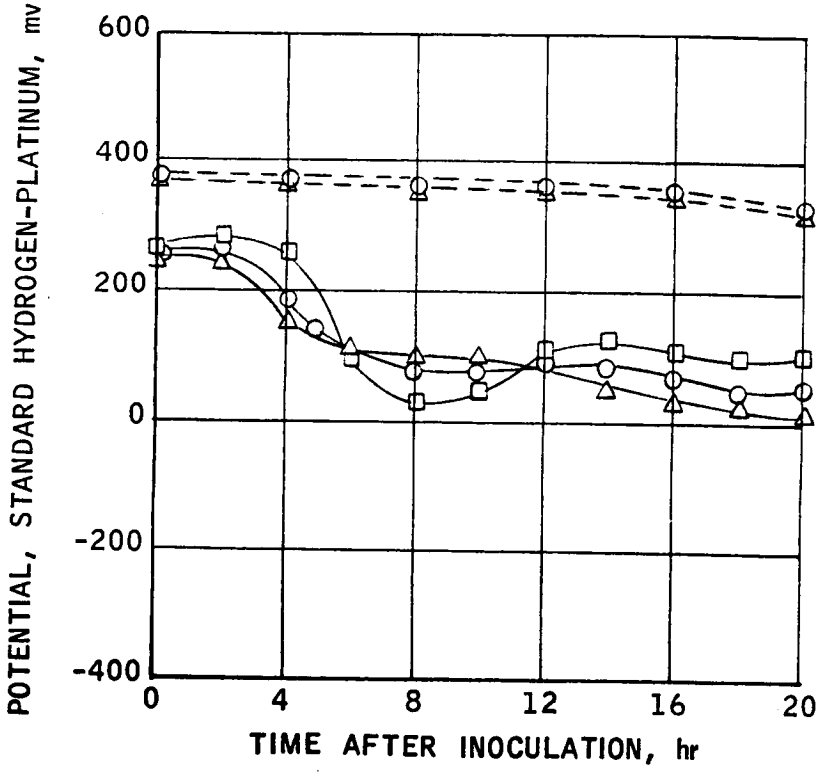
Figure 17

CHANGES OF POTENTIAL PRODUCED BY B. MEGATERIUM, S. FAECALIS, AND S. MARCESCENS IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 60

INOCULA: ALL 18 hr CULTURES GROWN AT 37°C

INOCULA SIZES: BACILLUS MEGATERIUM, APPROX. 5×10^6 ORGANISMS/ml
STREPTOCOCCUS FAECALIS, APPROX. 10^7 ORGANISMS/ml
SERRATIA MARCESCENS, APPROX. 10^7 ORGANISMS/ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION
ASEPTIC

- - - - - - - UNINOCULATED

——————— INOCULATED

TESTS:

○ = BACILLUS MEGATERIUM

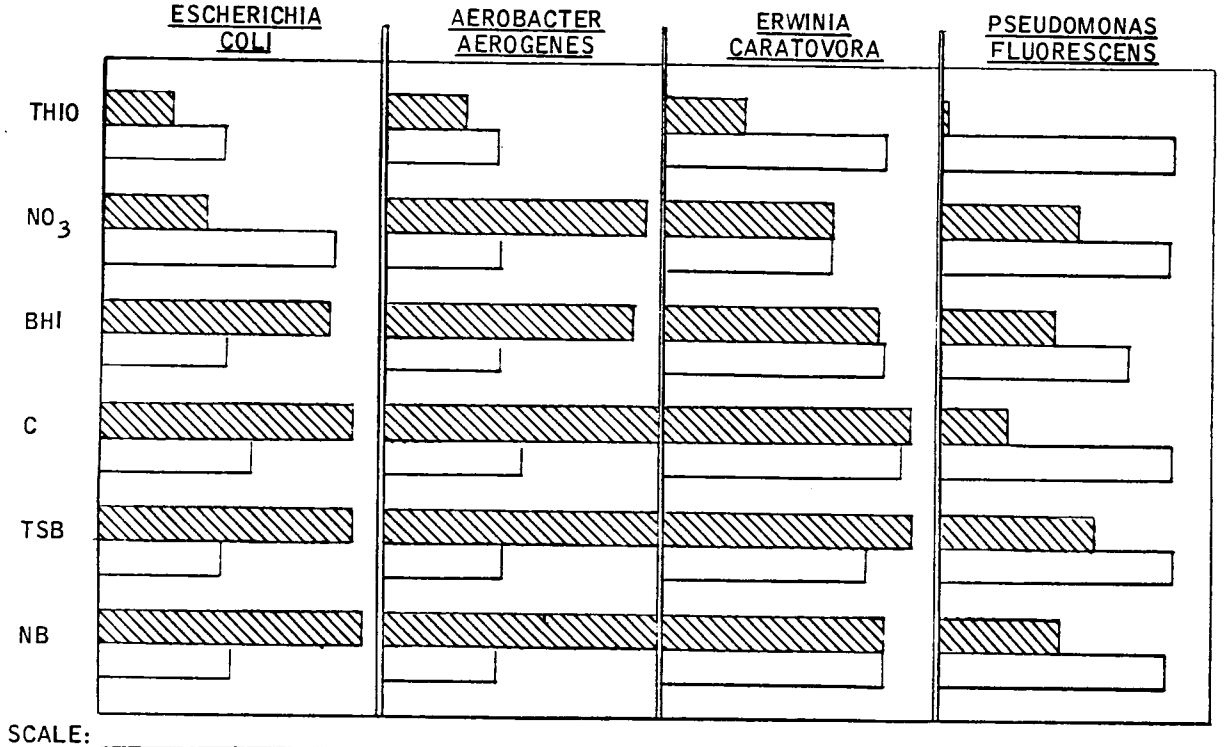
□ = STREPTOCOCCUS FAECALIS

△ = SERRATIA MARCESCENS

*DESCRIBED IN TABLE I

Figure 18

TOTAL POTENTIAL CHANGES PRODUCED BY MICROBES IN VARIOUS MEDIA* AND ASSOCIATED TIME INTERVALS



THIO = FLUID THIOGLYCOLLATE BROTH

NO_3 = NITRATE BROTH

BHI = BRAIN HEART INFUSION

C = SYNTHETIC MEDIUM C

TSB = TRYPTICASE SOY BROTH

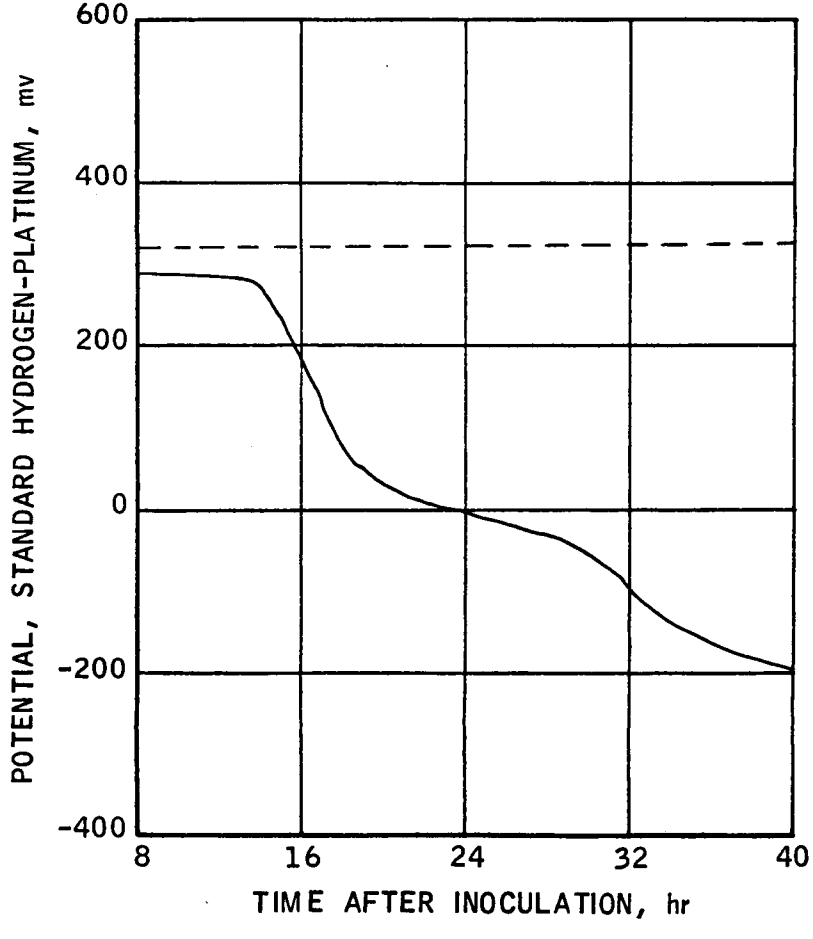
NB = NUTRIENT BROTH

*DESCRIBED IN TABLE T

Figure 19

CHANGES IN POTENTIAL PRODUCED BY SOIL SAMPLE* IN TRYPTICASE SOY BROTH**

INOCULUM: 1 gram SOIL IN 10 ml STERILE, DEIONIZED WATER, SHAKEN 10 minutes
INOCULUM SIZE: 1 ml ABOVE SUSPENSION IN 30 ml OF MEDIA** (APPROXIMATELY).



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT
NO AGITATION

ASEPTIC

UNINOCULATED (AVERAGE OF 5 CURVES)

INOCULATED (AVERAGE OF 5 CURVES)

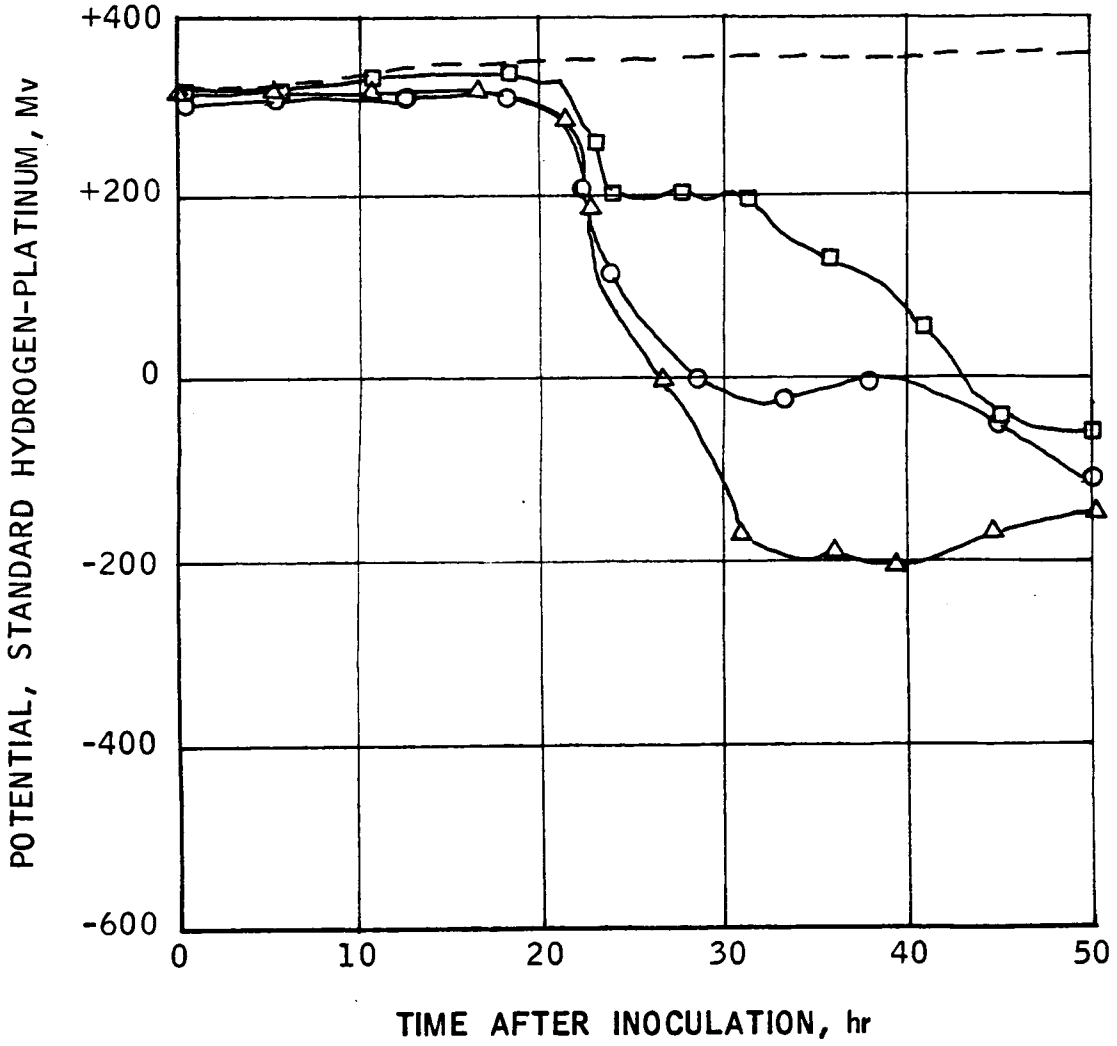
*DESCRIBED IN TABLE V

** DESCRIBED IN TABLE I

Figure 20

CHANGES IN POTENTIAL PRODUCED BY SOIL SAMPLES* IN TRYPTICASE SOY BROTH**

INOCULUM: 0.1 gm SOIL IN 30 ml MEDIUM



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION
ASEPTIC

---- UNINOCULATED

——— INOCULATED

△ = SOIL SAMPLE 9

□ = SOIL SAMPLE 2

○ = SOIL SAMPLE 1

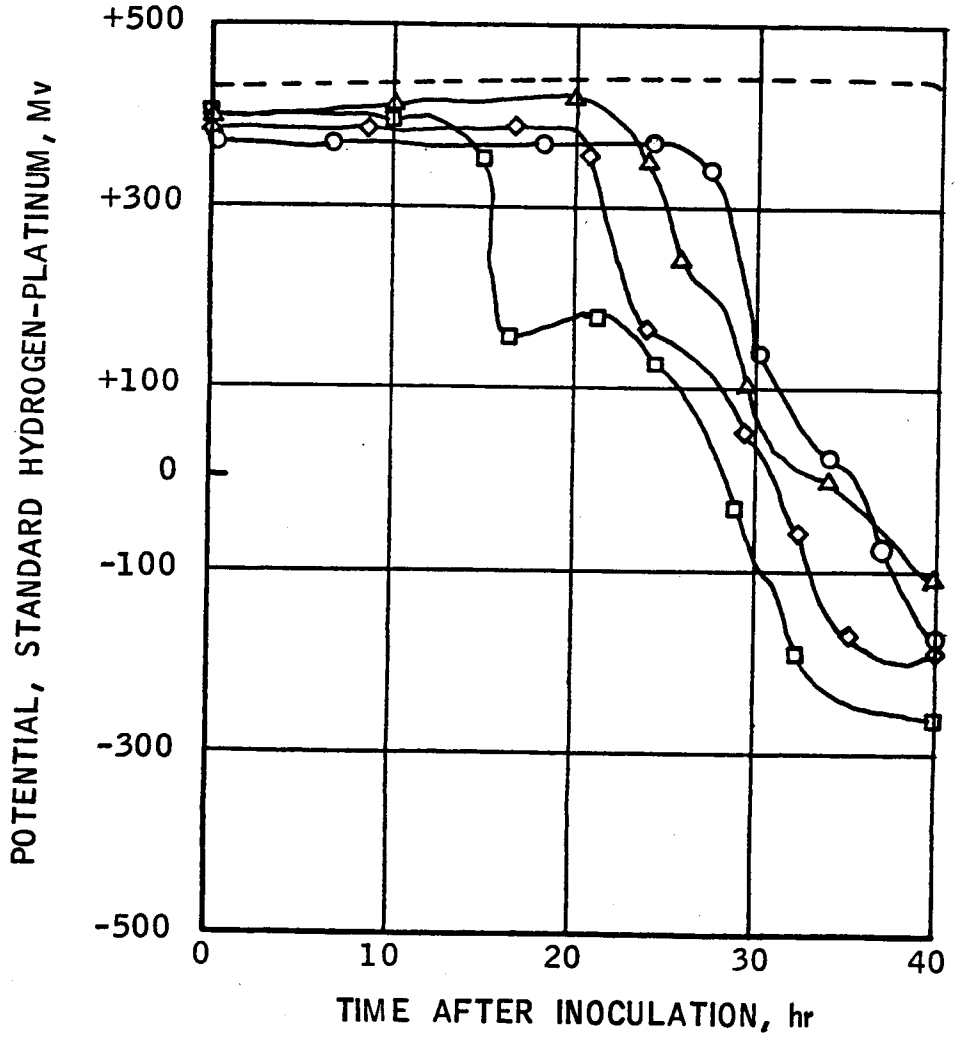
*DESCRIBED IN TABLE I

**DESCRIBED IN TABLE II

Figure 21

CHANGES IN POTENTIAL PRODUCED BY SOIL SAMPLES* IN TRYPTICASE SOY BROTH**

INOCULUM: 0.1 gm SOIL IN 30 ml MEDIUM



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION
ASEPTIC

- - - - UNINOCULATED
——— INOCULATED

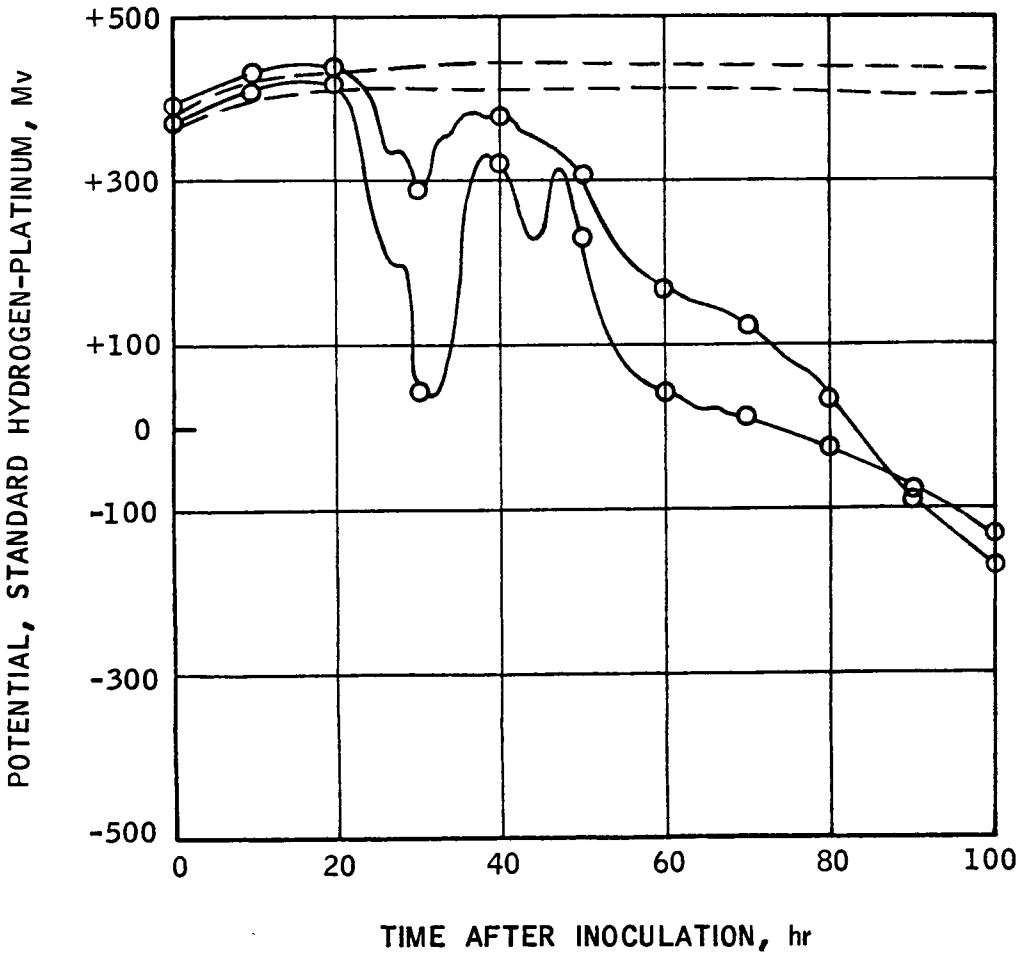
△ = SOIL SAMPLE 9
□ = SOIL SAMPLE 3
○ = SOIL SAMPLE 5
◇ = SOIL SAMPLE 6

*DESCRIBED IN TABLE I
**DESCRIBED IN TABLE II

Figure 22

CHANGES IN POTENTIAL PRODUCED BY GARDEN SOIL IN MEDIUM SELECTIVE FOR AZOTOBACTER*

INOCULUM: 0.3 gm SOIL IN 30 ml MEDIUM



TEST CONDITIONS:

TEMPERATURE = 24 - 25 °C
ATMOSPHERE = AMBIENT
NO AGITATION
ASEPTIC

– – – – UNINOCULATED
——— INOCULATED

*DESCRIBED IN TABLE I

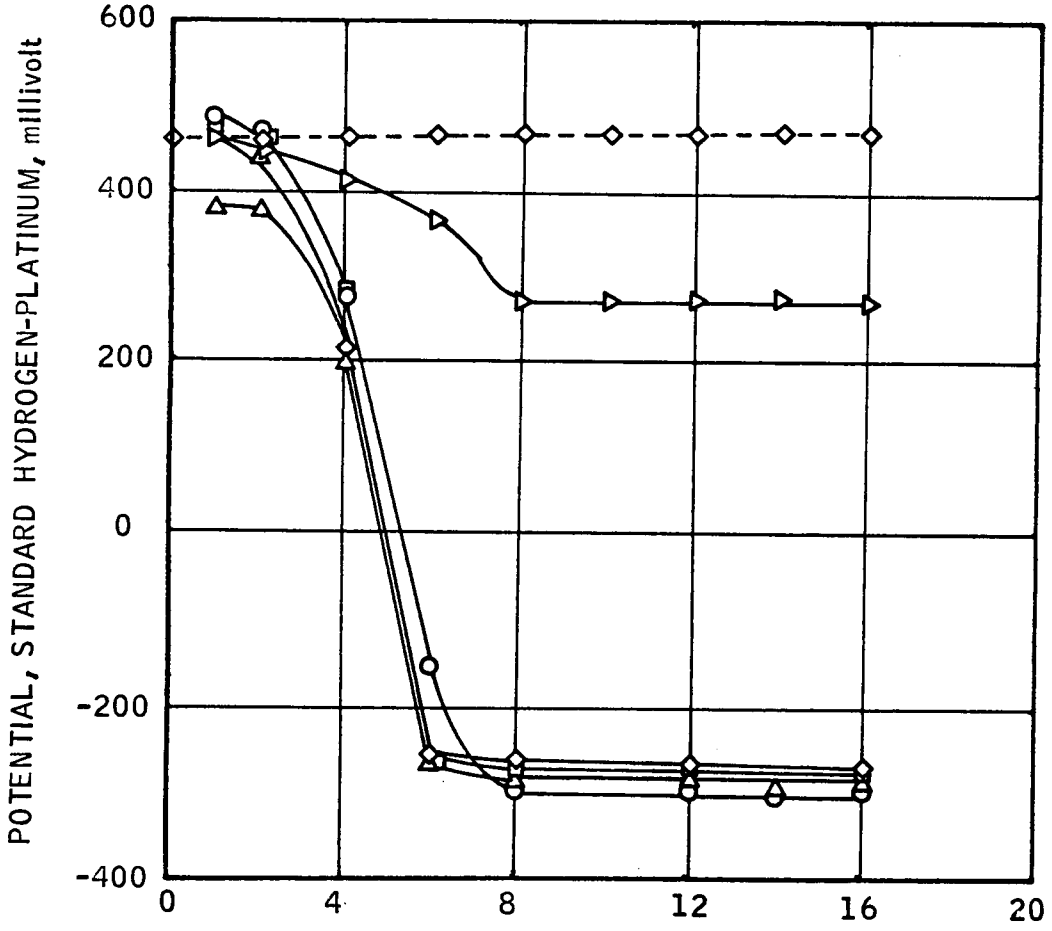
Figure 23

EFFECT OF CONCENTRATION OF SYNTHETIC MEDIUM C* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENT NO. 22

INOCULUM: E. COLI, 22 HR CULTURE GROWN AT 37°C

INOCULUM SIZE: APPROXIMATELY 8×10^8 ORGANISMS IN 31 ML
DILUTION REFERS TO RATIO OF VOLUME OF SYNTHETIC MEDIUM: TOTAL VOLUME
DILUTIONS WERE MADE WITH 0.9% SALINE SOLUTION



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

---- UNINOCULATED
—— INOCULATED

DILUTIONS

△ = 1:1 (FULL STRENGTH)
○ = 1:2
◇ = 1:3
□ = 1:4
▷ = 1:5

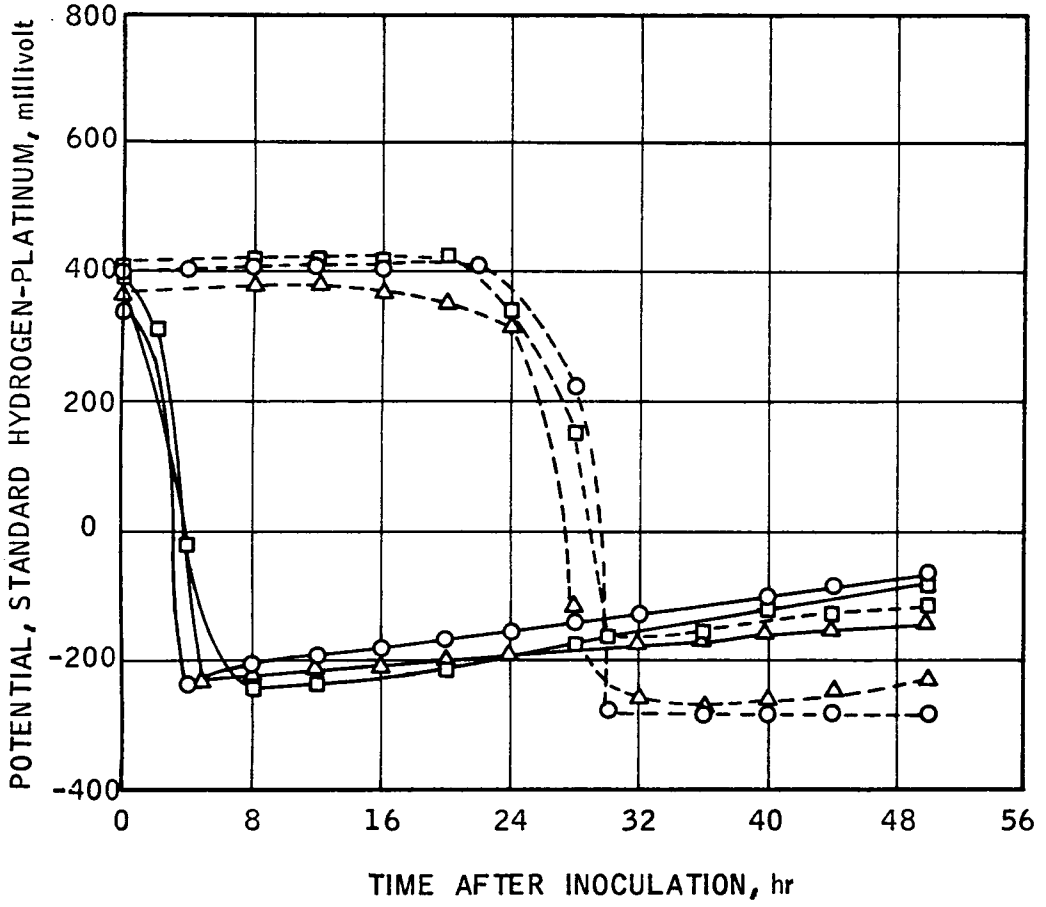
*DESCRIBED IN TABLE I

Figure 24

EFFECT OF CONCENTRATION OF NUTRIENT BROTH* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENT NO. 28B

INOCULUM: E. COLI, 18 HR CULTURE GROWN AT 37°C
INOCULUM SIZE: APPROXIMATELY 2×10^9 ORGANISMS IN 31 ML
MEDIUM: DOUBLE STRENGTH NUTRIENT BROTH SOLUTION
DILUTIONS: FULL STRENGTH MADE WITH DISTILLED WATER, HALF-STRENGTH WITH 0.9% SALINE



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
NON-STERILE

---- UNINOCULATED
—— INOCULATED

NUTRIENT CONCENTRATION

△ = DOUBLE STRENGTH
○ = FULL STRENGTH
□ = HALF STRENGTH

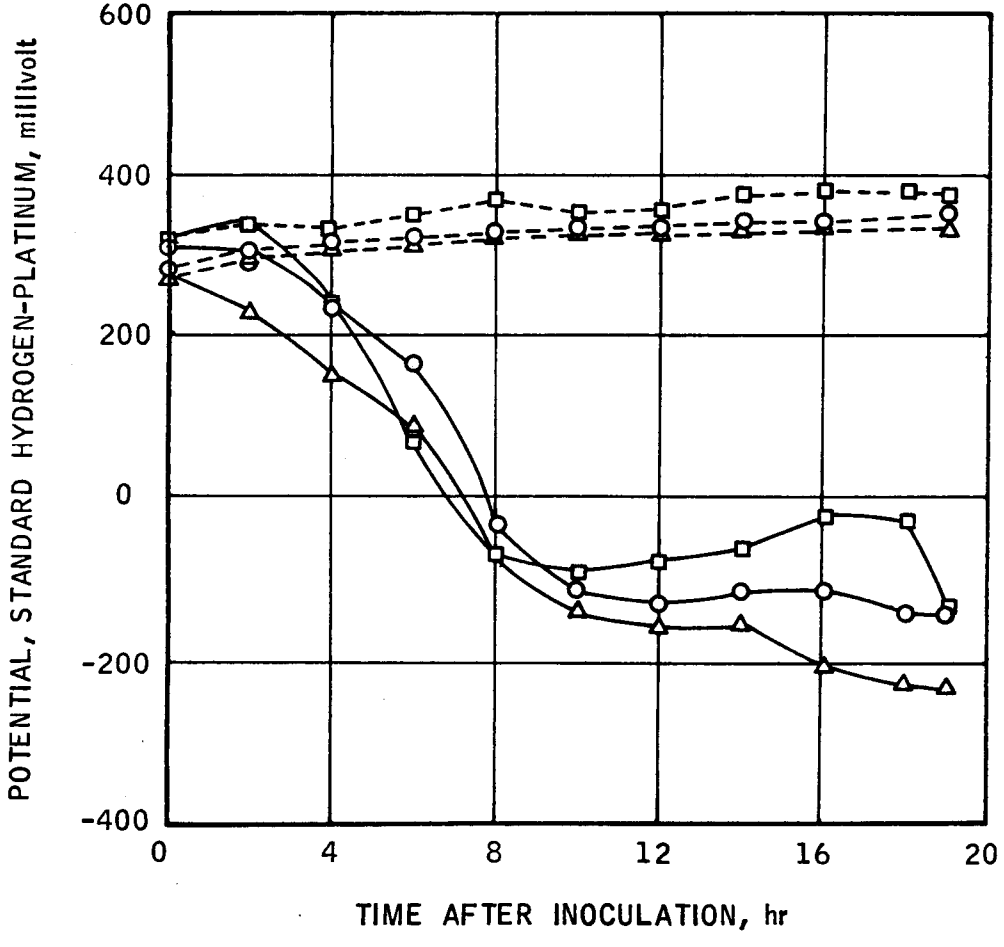
*DESCRIBED IN TABLE I

Figure 25

EFFECT OF CONCENTRATION OF NUTRIENT BROTH* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENT NO. 30B

INOCULUM: E. COLI, 18 HR CULTURE GROWN AT 37°C
INOCULUM SIZE: APPROXIMATELY 10^9 ORGANISMS IN 31 ML
MEDIUM: DOUBLE STRENGTH NUTRIENT BROTH SOLUTION
DILUTIONS: FULL STRENGTH MADE WITH DISTILLED WATER, HALF STRENGTH WITH 0.9% SALINE



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

---- UNINOCULATED
—— INOCULATED

NUTRIENT CONCENTRATION

△ = DOUBLE STRENGTH
○ = FULL STRENGTH
□ = HALF STRENGTH

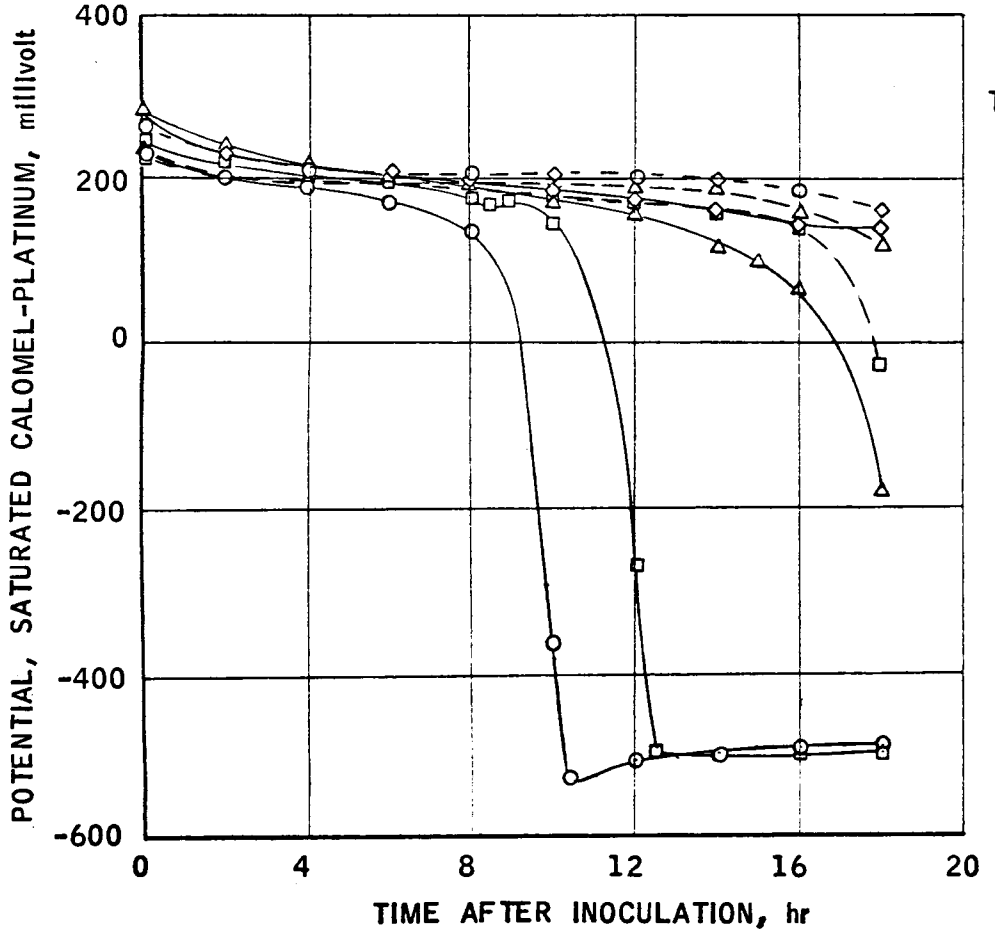
*DESCRIBED IN TABLE I

Figure 26

EFFECT OF CONCENTRATION OF NUTRIENT BROTH* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENT No. 10

INOCULUM SIZE: MODERATE (APPROX. 10^8 CELLS IN 31 ml)
DILUTION REFERS TO RATIO OF VOLUME OF NUTRIENT BROTH: TOTAL VOLUME
DILUTIONS WERE MADE WITH 0.9% SALINE SOLUTION



TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT, NO AGITATION AFTER INOCULATION

- - - - UNINOCULATED
———— INOCULATED

DILUTION
○ = 1:2
□ = 1:2.5
△ = 1:3
◇ = 1:3.5

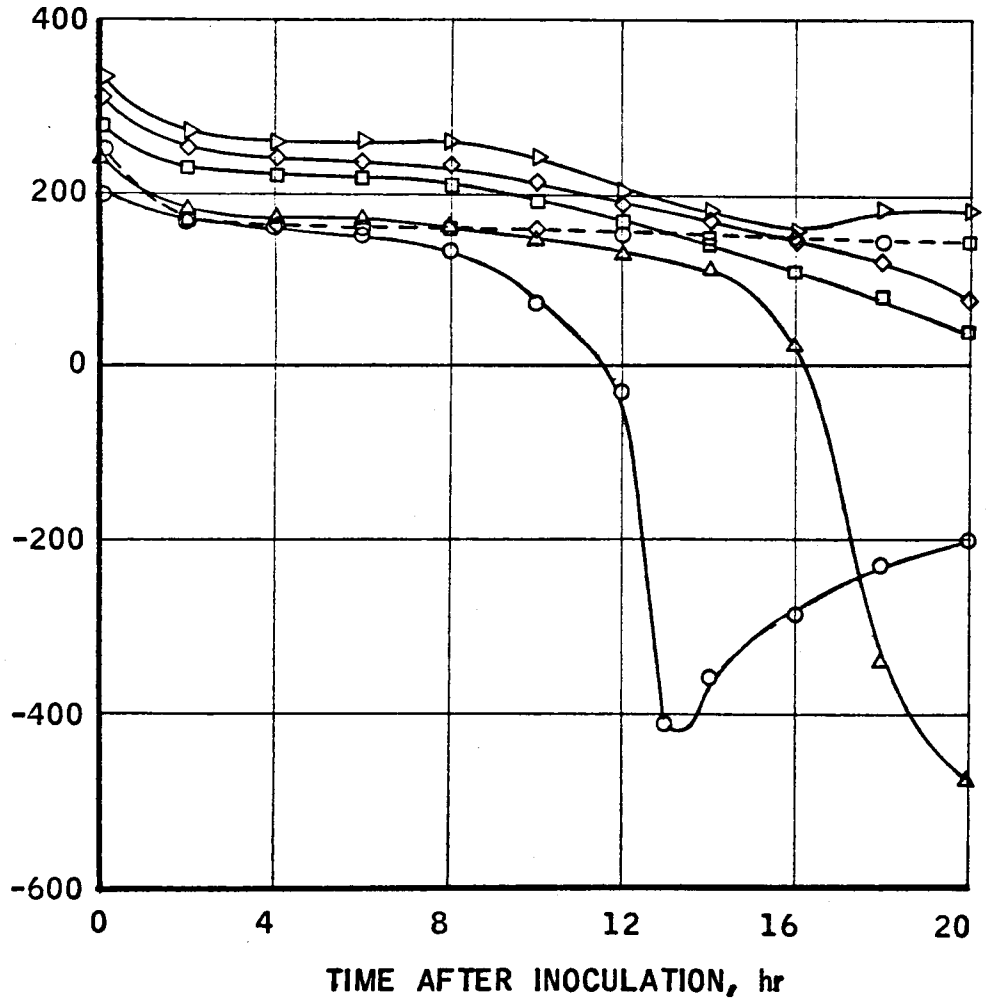
*DESCRIBED IN TABLE I

Figure 27

EFFECT OF CONCENTRATION OF NUTRIENT BROTH* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENT No. 7

INOCULUM SIZE: LIGHT (APPROX. 10^7 CELLS IN 31 ml)
DILUTION RATIO REFERS TO VOLUME OF NUTRIENT BROTH: TOTAL VOLUME
DILUTIONS WERE MADE WITH 0.9% SALINE SOLUTION



TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT, NO AGITATION AFTER INOCULATION

----- UNINOCULATED
——— INOCULATED

DILUTION

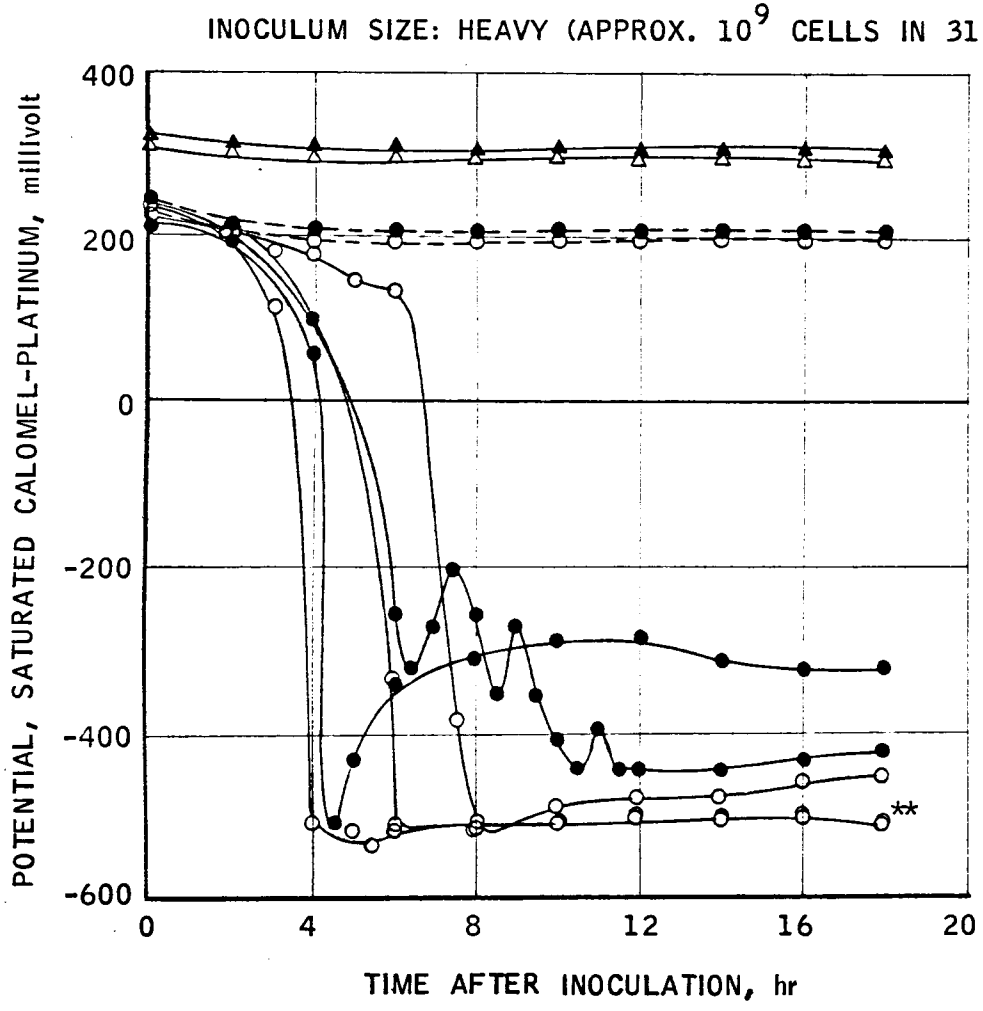
△ = 1:1 (FULL STRENGTH)
○ = 1:2
□ = 1:4
◇ = 1:8
▷ = 1:16

*DESCRIBED IN TABLE I

Figure 28

EFFECT OF ATMOSPHERIC CONDITIONS ON POTENTIAL CHANGE
PRODUCED BY E. COLI IN NUTRIENT BROTH* AND IN SALINE SOLUTION

EXPERIMENT No. 12



TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT, NO AGITATION AFTER INOCULATION

– – – – UNINOCULATED
——— INOCULATED

△ = 0.9% SALINE SOLUTION, ANAEROBIC
▲ = 0.9% SALINE SOLUTION, AEROBIC
○ = NUTRIENT BROTH, ANAEROBIC
● = NUTRIENT BROTH, AEROBIC

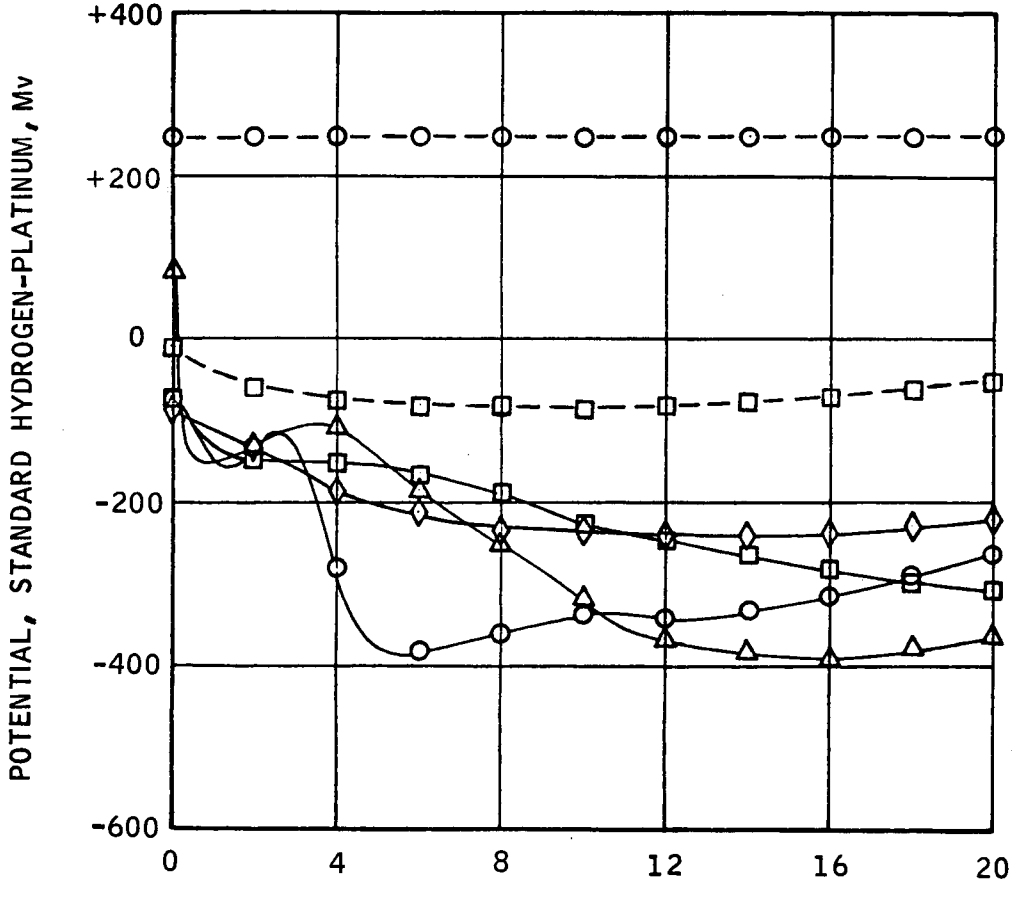
*1/2 NORMAL CONCENTRATION OF INGREDIENTS (SEE TABLE I)

** NOTE THAT 2 LINES CONVERGE

Figure 29

CHANGES IN POTENTIAL PRODUCED BY CL. SPOROGENES AND E. COLI IN TRYPTICASE SOY BROTH* AND THIOGLYCOLLATE BROTH*

INOCULUM: APPROXIMATELY 10^7 MICROORGANISMS/MILLILITER



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
NO AGITATION
ASEPTIC
ATMOSPHERE = AIR OVER THIOGLYCOLLATE BROTH, HELIUM OVER TRYPTICASE SOY BROTH

- TRYPTICASE SOY BROTH, UNINOCULATED
- THIOGLYCOLLATE BROTH, UNINOCULATED
- TRYPTICASE SOY BROTH, E. COLI
- TRYPTICASE SOY BROTH CL. SPOROGENES
- THIOGLYCOLLATE BROTH, CL. SPOROGENES
- THIOGLYCOLLATE BROTH, E. COLI

*DESCRIBED IN TABLE I

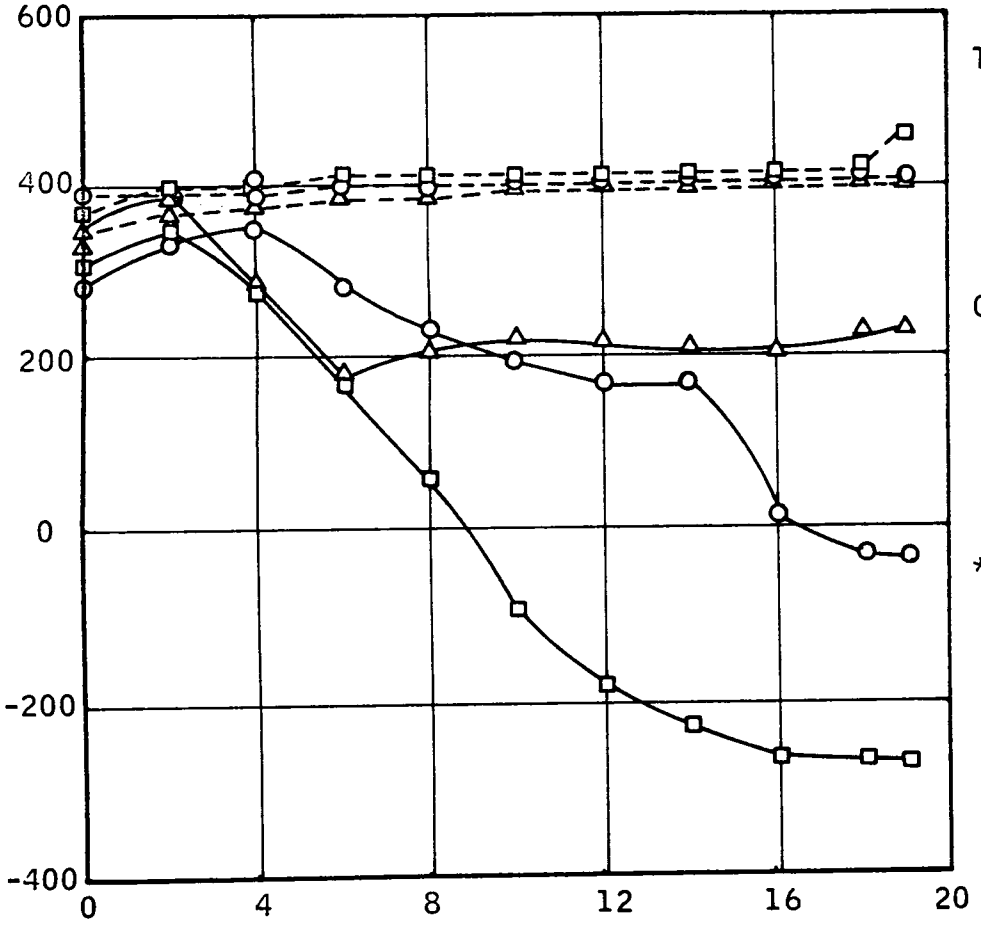
Figure 30

EFFECT OF CONCENTRATION OF GLUCOSE IN SYNTHETIC MEDIUM C* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENT NO. 30A

INOCULUM: E. COLI, 18 HR CULTURE GROWN AT 37°C

INOCULUM SIZE: APPROX. 10^9 ORGANISMS IN 31 ML



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

GLUCOSE CONCENTRATION

△ = 0.01%
○ = 0.1%
□ = 1.0%

*DESCRIPTION IN TABLE I

---- UNINOCULATED
—— INOCULATED

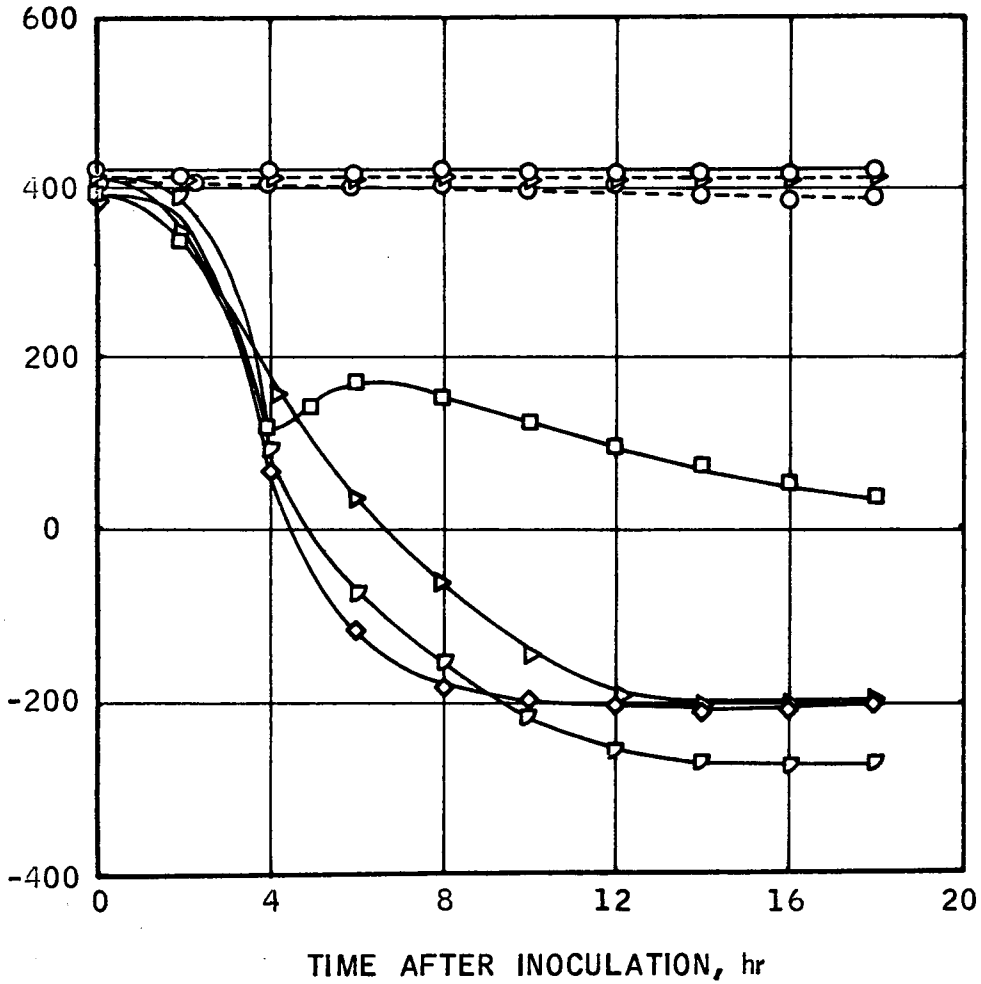
Figure 31

EFFECT OF CONCENTRATION OF GLUCOSE IN SYNTHETIC MEDIUM C* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENT NO. 25

INOCULUM: E. COLI, 18 HR CULTURE GROWN AT 37°C

INOCULUM SIZE: APPROX. 2×10^9 ORGANISMS IN 31 ML



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

GLUCOSE CONCENTRATION

○ = 0.0001%
□ = 0.01%
▽ = 0.1%
◇ = 1.0%
▷ = 5.0%

*DESCRIBED IN TABLE I

---- UNINOCULATED
—— INOCULATED

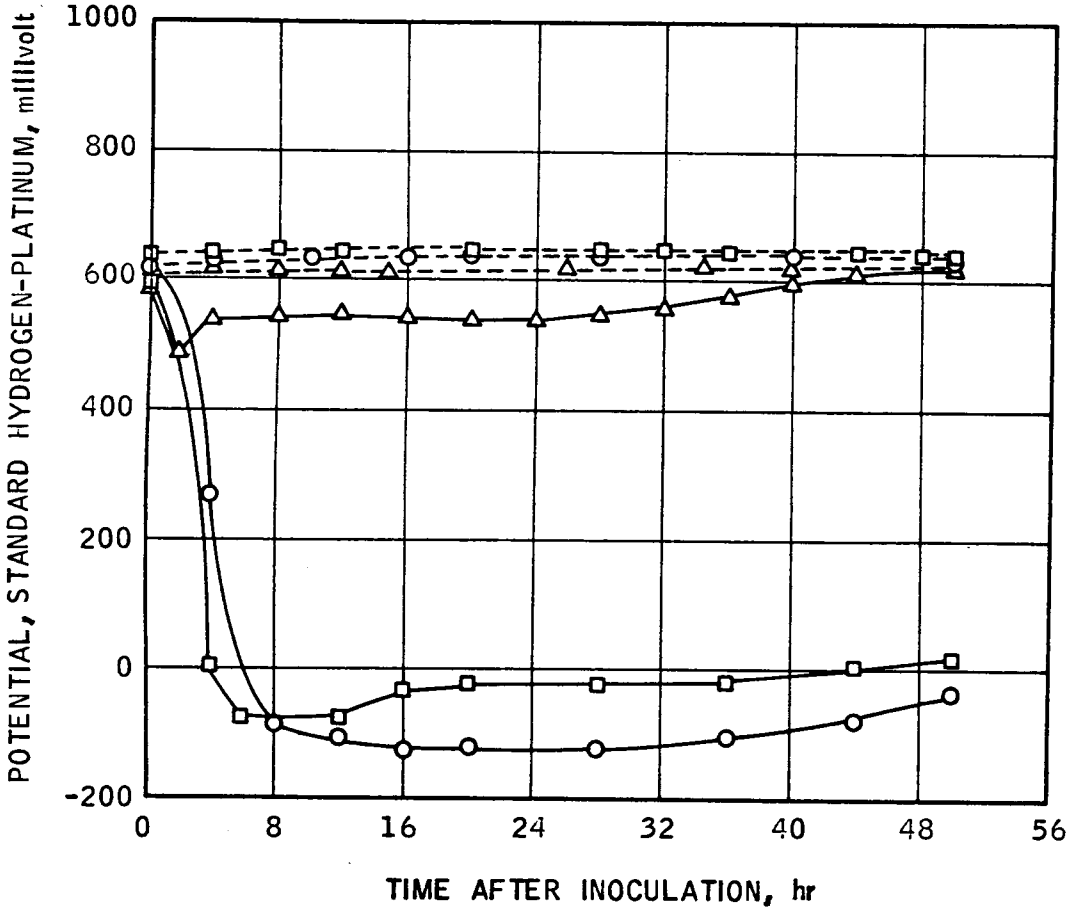
Figure 32

EFFECT OF CONCENTRATION OF GLUCOSE IN SYNTHETIC MEDIUM C* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENT NO. 28A

INOCULUM: E. COLI, 18 HR CULTURE GROWN AT 37°C

INOCULUM SIZE: APPROX. 4×10^9 ORGANISMS IN 31 ML



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

---- UNINOCULATED

—— INOCULATED

GLUCOSE CONCENTRATION

△ = 0.01%

○ = 0.1%

□ = 1.0%

*DESCRIBED IN TABLE I

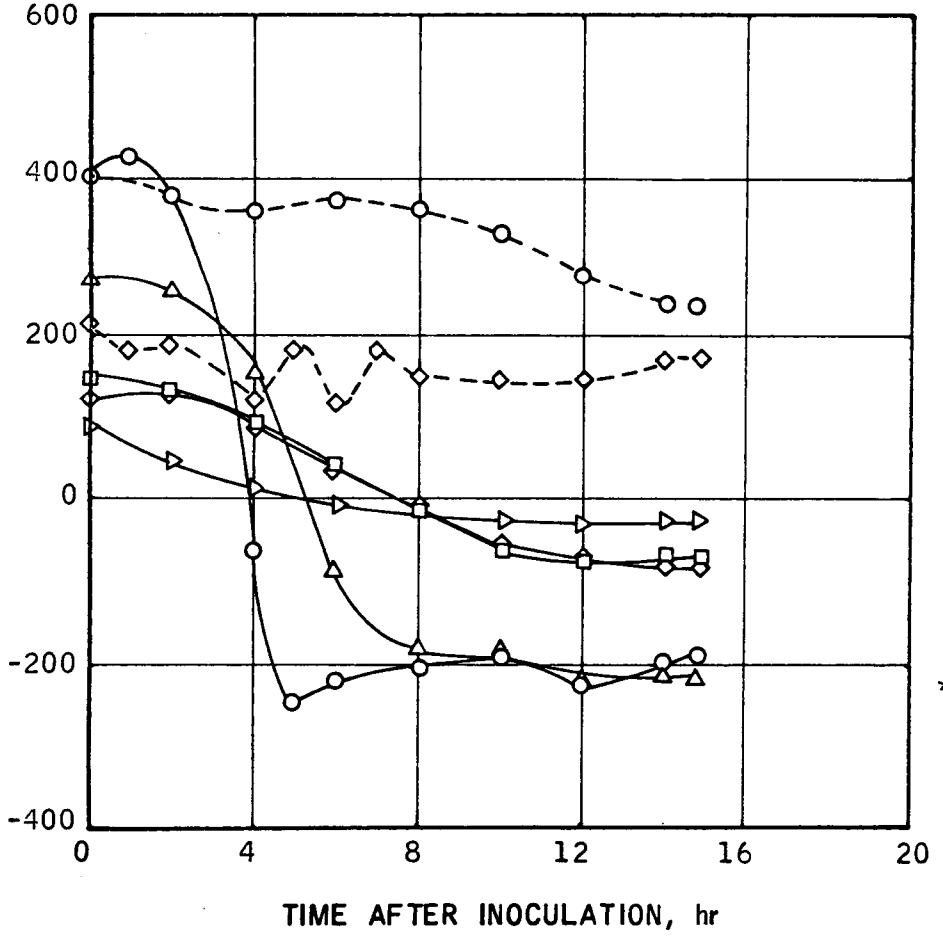
Figure 33

EFFECT OF THIOGLYCOLLATE CONCENTRATION ON POTENTIAL CHANGE PRODUCED BY *E. COLI* IN NUTRIENT BROTH*

EXPERIMENT No. 29

INOCULUM: *E. COLI*, 24 HR CULTURE GROWN AT 37° C, IN SYNTHETIC MEDIUM C

INOCULUM SIZE: APPROX. 10^{10} ORGANISMS IN 31 ML



TEST CONDITIONS:

TEMPERATURE = 24 - 25° C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

---- UNINOCULATED

—— INOCULATED

THIOGLYCOLLATE CONCENTRATION

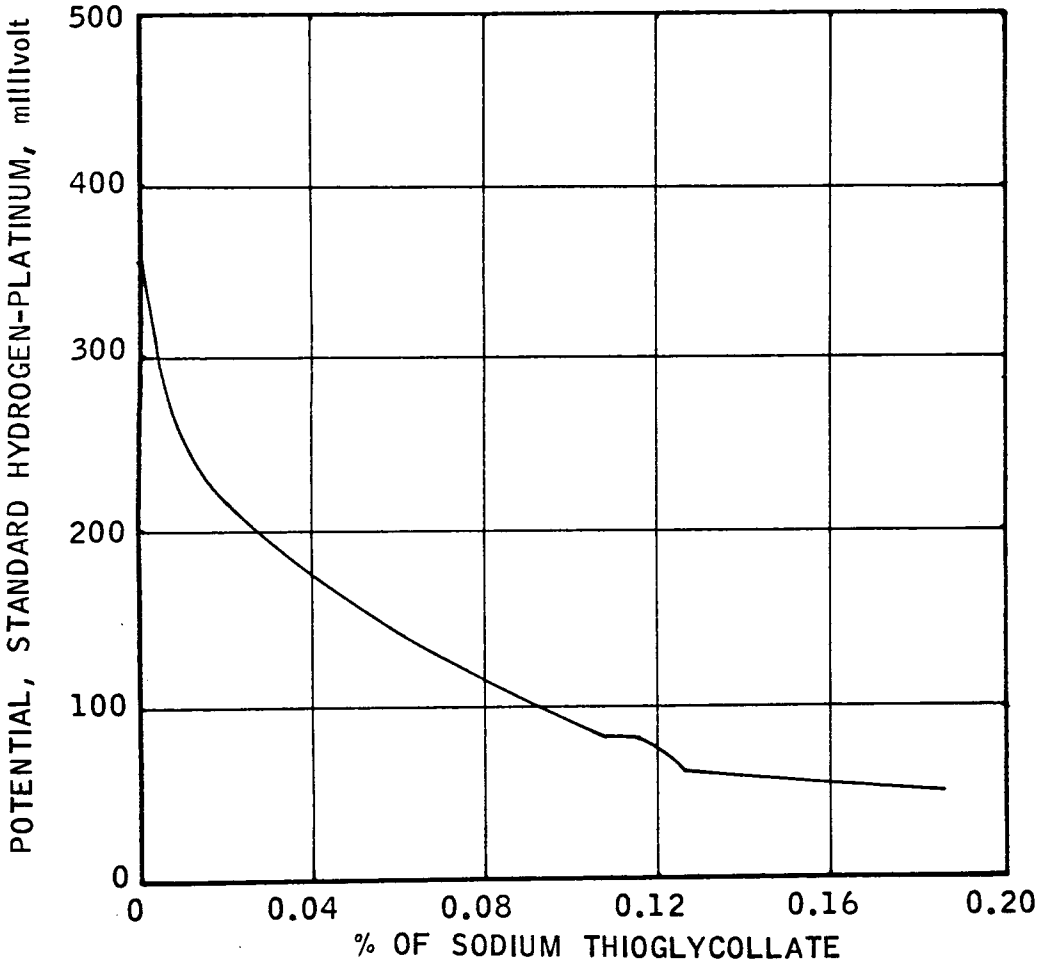
○= 0.0% (NO THIOGLYCOLLATE)
△= 0.005%
□= 0.05%
◇= 0.1%
▷= 0.5%

*DESCRIBED IN TABLE 1

Figure 34

POTENTIAL CHANGE PRODUCED BY SODIUM THIOGLYCOLLATE IN SYNTHETIC MEDIUM C*

EXPERIMENT No. 33



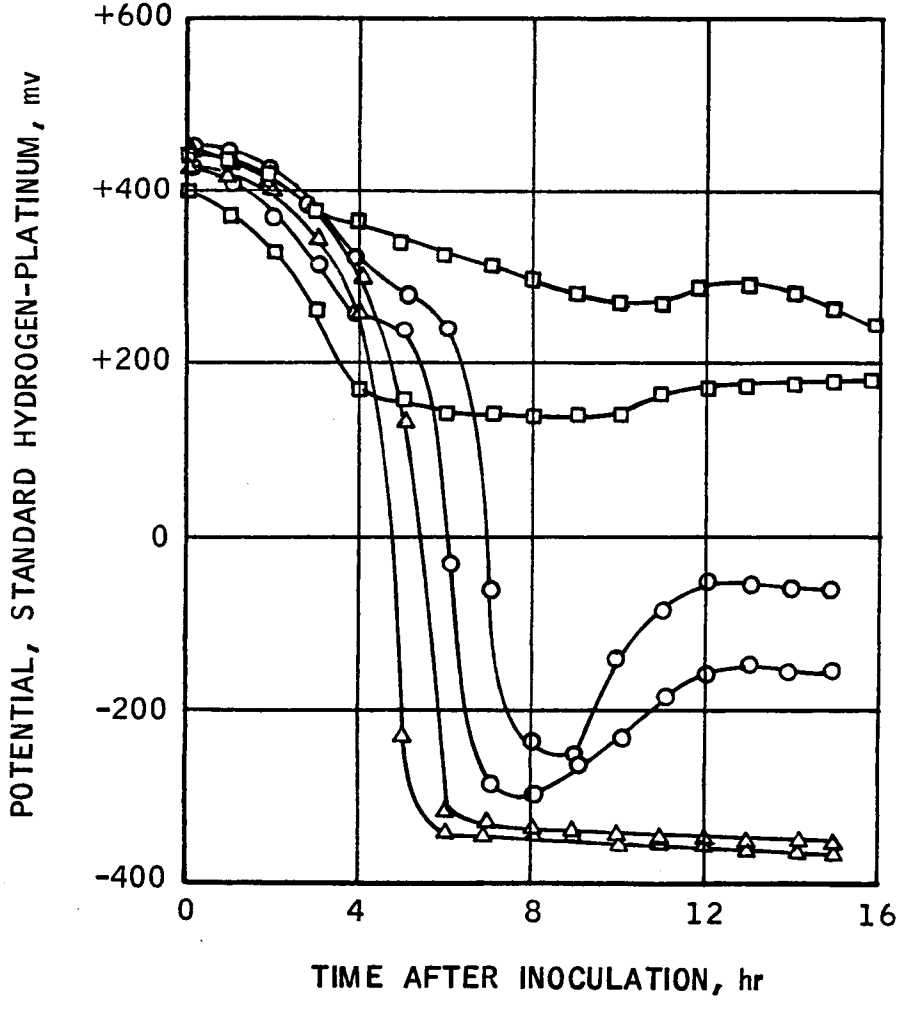
*DESCRIBED IN TABLE I

Figure 35

CHANGES IN POTENTIAL PRODUCED BY E. COLI IN NUTRIENT BROTH* WITH DIFFERENT CONCENTRATIONS OF NITRATE

INOCULA: E. COLI, 6 hr CULTURE GROWN AT 37°C. FROM A HEAVY INOCULUM

INOCULA SIZES: APPROX. 3×10^6 MICROORGANISMS/ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C.
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

POTENTIAL RANGE LIMITS OF NITRATE CONCENTRATIONS

□ = 0.1 AND 0.05%
○ = 0.01%
△ = NO NITRATE

*DESCRIBED IN TABLE I

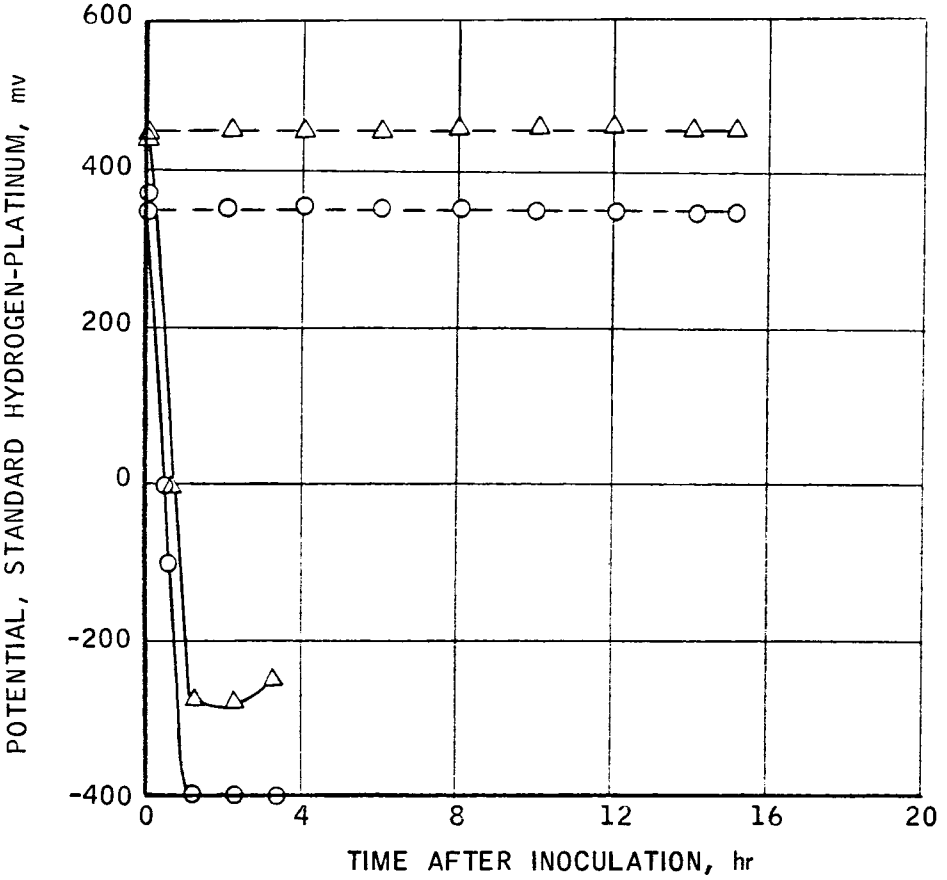
Figure 36

CHANGE OF POTENTIAL WITH *E. COLI* IN STANDARD AND MODIFIED SYNTHETIC MEDIUM C*

EXPERIMENT NO. 44

INOCULUM: 24 hr CULTURE GROWN AT 37°C

INOCULUM SIZE: APPROX. 2×10^{10} ORGANISMS IN 31 ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
NON-STERILE

– – – – UNINOCULATED

——— INOCULATED

MEDIUM

○ = SYNTHETIC MEDIUM C

△ = SYNTHETIC MEDIUM C BUT WITH 1% RATHER THAN 0.1% GLUCOSE AND WITH ONLY 1/10th STANDARD CONCENTRATION OF Na_2HPO_4

*DESCRIBED IN TABLE I

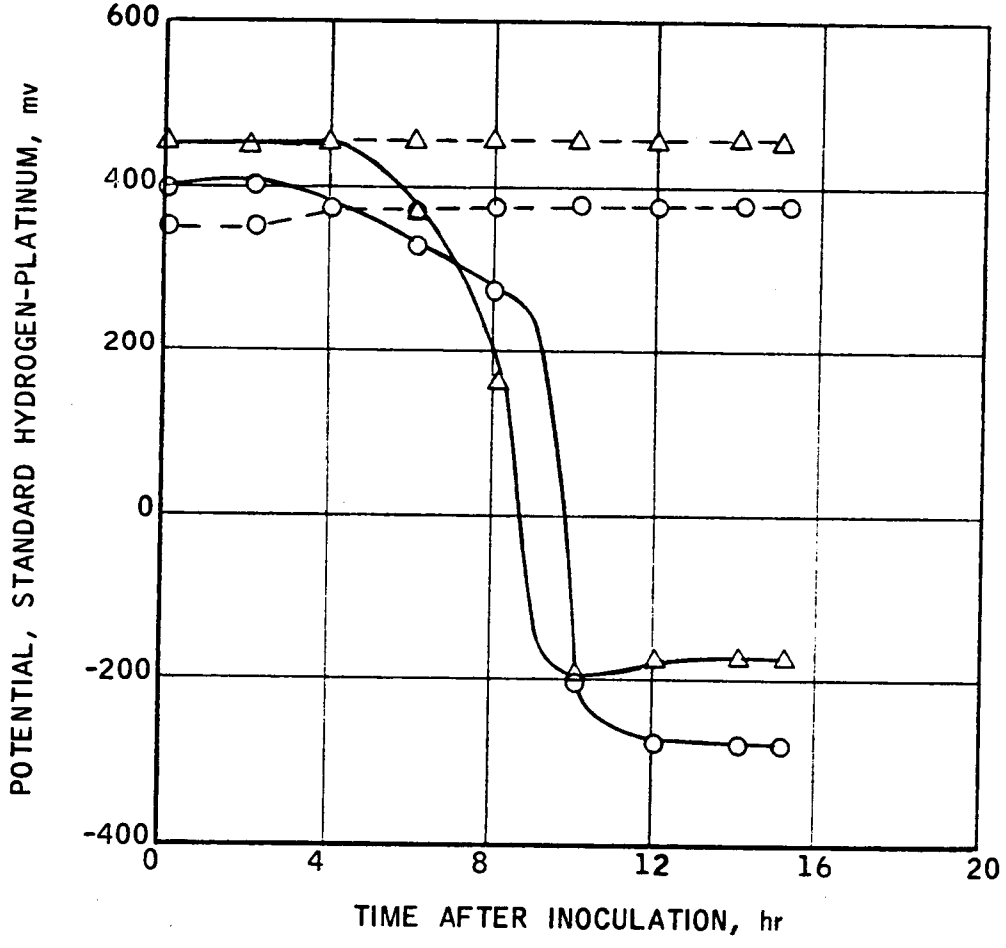
Figure 37

CHANGE OF POTENTIAL WITH E. COLI IN STANDARD AND MODIFIED SYNTHETIC MEDIUM C*

EXPERIMENT NO. 45

INOCULUM: 48 hr CULTURE GROWN AT 37°C

INOCULUM SIZE: APPROX. 10^8 ORGANISMS IN 31 ml



TEST CONDITIONS:

TEMPERATURE = 24-25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
NON-STERILE

- - - - - UNINOCULATED

——— INOCULATED

MEDIUM:

○ = SYNTHETIC MEDIUM C

△ = SYNTHETIC MEDIUM C BUT WITH 1% RATHER THAN 0.1% GLUCOSE AND WITH ONLY 1/10th STANDARD CONCENTRATION OF Na_2PO_4

* DESCRIBED IN TABLE I

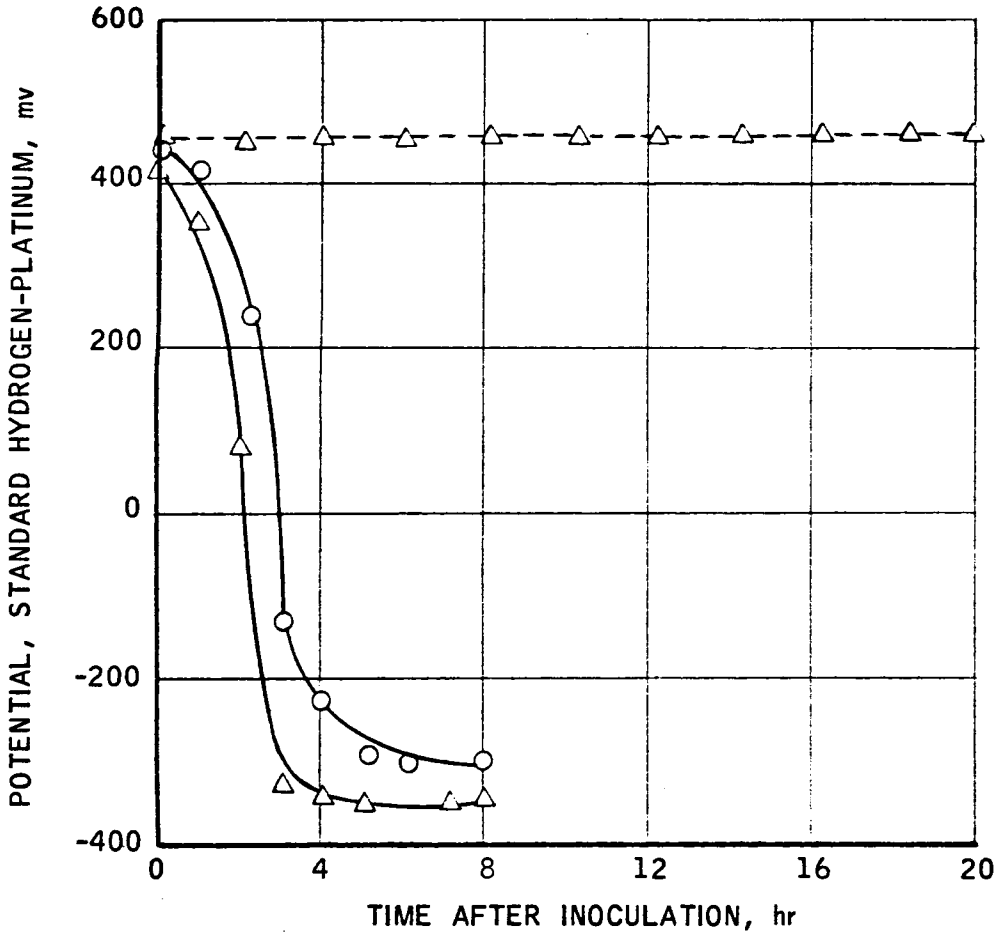
Figure 38

POTENTIAL CHANGE PRODUCED BY E. COLI IN SYNTHETIC MEDIUM C*WITH AND WITHOUT NITROGEN SOURCE

EXPERIMENT NO. 37

INOCULUM: 20 hr CULTURE GROWN AT 37° C

INOCULUM SIZE: APPROX. 10^{10} ORGANISMS IN 31 ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
NON-STERILE

----- UNINOCULATED

——— INOCULATED

NITROGEN SOURCE

△ = 2 g/l NH_4Cl

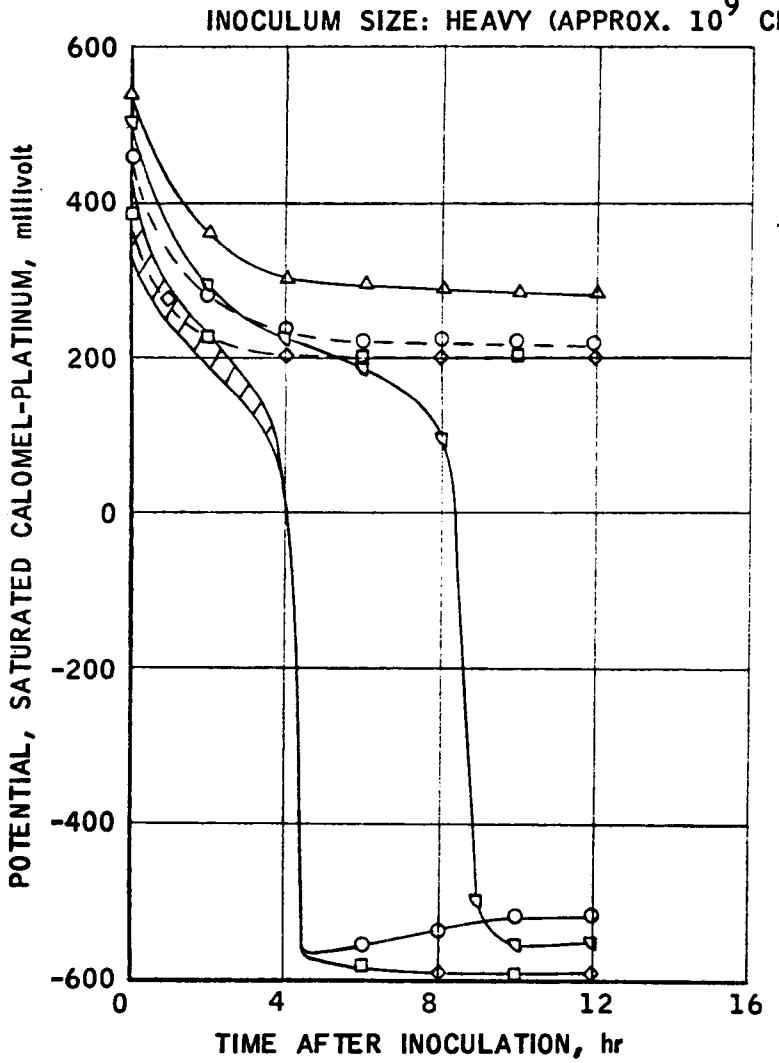
○ = NO NITROGEN SOURCE

* DESCRIBED IN TABLE I

Figure 39

EFFECT OF CONCENTRATION OF YEAST EXTRACT IN SYNTHETIC MEDIUM* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENT No. 13



TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT, NO AGITATION AFTER INOCULATION

— — — — — UNINOCULATED
———— INOCULATED

△ = 0.9% SALINE SOLUTION
○ = 0.1% YEAST EXTRACT IN SYNTHETIC MEDIUM
□ = 0.3% YEAST EXTRACT IN SYNTHETIC MEDIUM
◇ = 0.5% YEAST EXTRACT IN SYNTHETIC MEDIUM
◁ = 0% YEAST EXTRACT IN SYNTHETIC MEDIUM
▨ = 0.1%, 0.3%, AND 0.5% LINES CONVERGE

*SYNTHETIC MEDIUM IS DESCRIBED IN TABLE I

Figure 40

EFFECT OF CONCENTRATION OF YEAST EXTRACT IN SYNTHETIC MEDIUM* ON POTENTIAL CHANGE PRODUCED BY E. COLI

EXPERIMENTS 20 AND 21

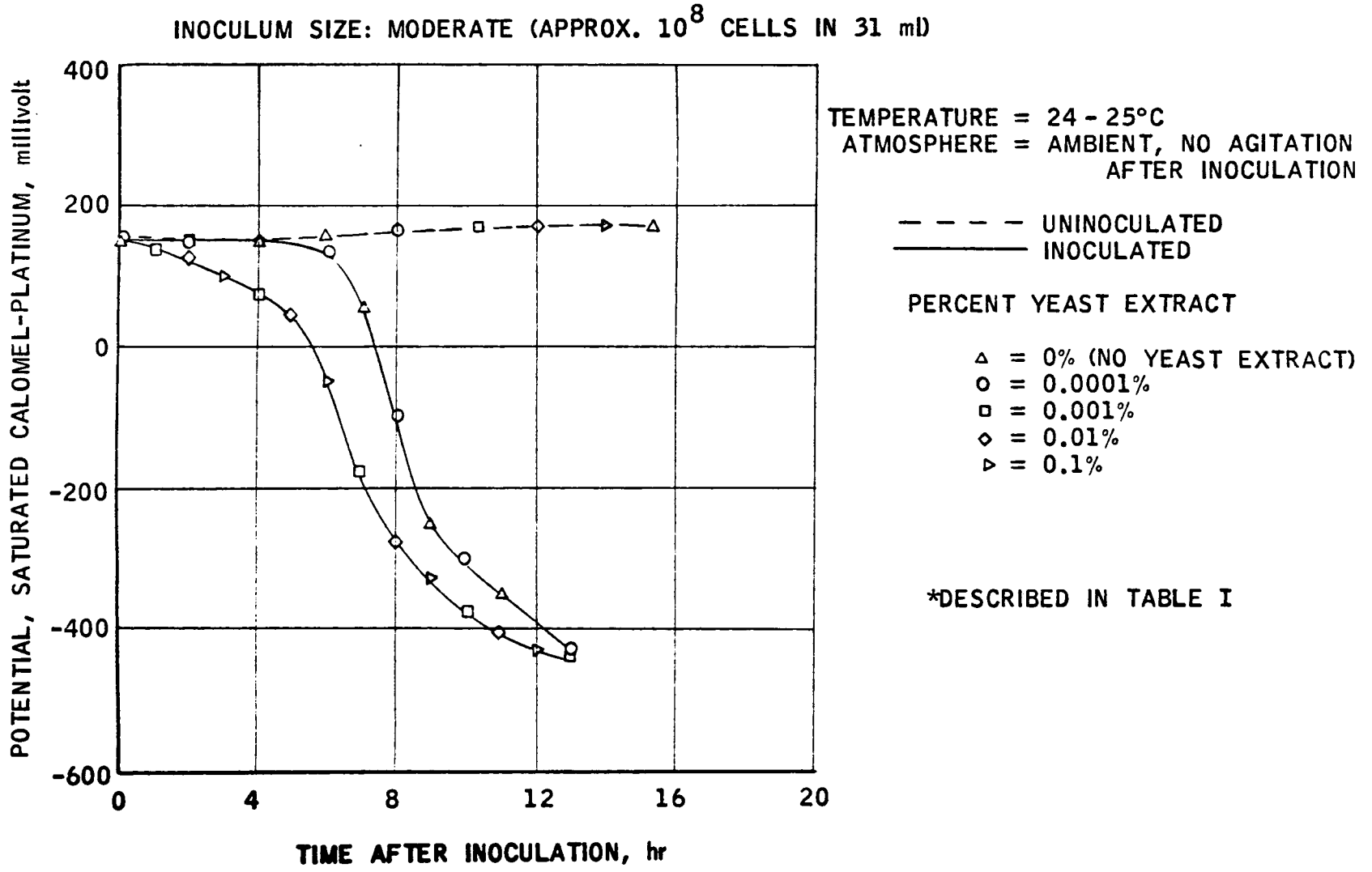


Figure 41

EFFECT OF CONCENTRATION OF E. COLI ON POTENTIAL CHANGE IN NUTRIENT BROTH*

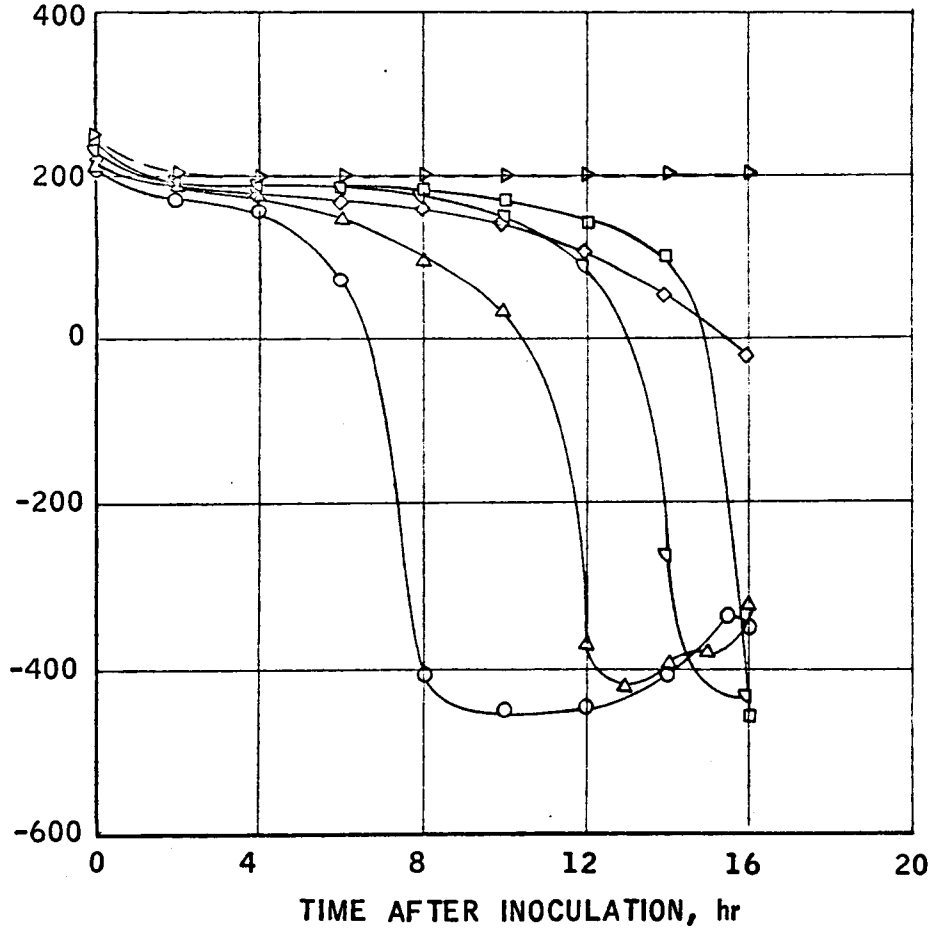
EXPERIMENT No. 8

INOCULUM SIZE: 1.0 ml OF VARIOUS DILUTIONS IN 31 ml TOTAL VOLUME

1.1 CONCENTRATION IS APPROXIMATELY 10^{10} CELLS IN 31 ml

DILUTION REFERS TO RATIO OF CONCENTRATED CELLS: TOTAL VOLUME OF SALINE DILUENT

DILUTIONS WERE MADE WITH 0.9% SALINE SOLUTION



TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT, NO. AGITATION AFTER INOCULATION

– – – – – – UNINOCULATED
———— INOCULATED

DILUTION

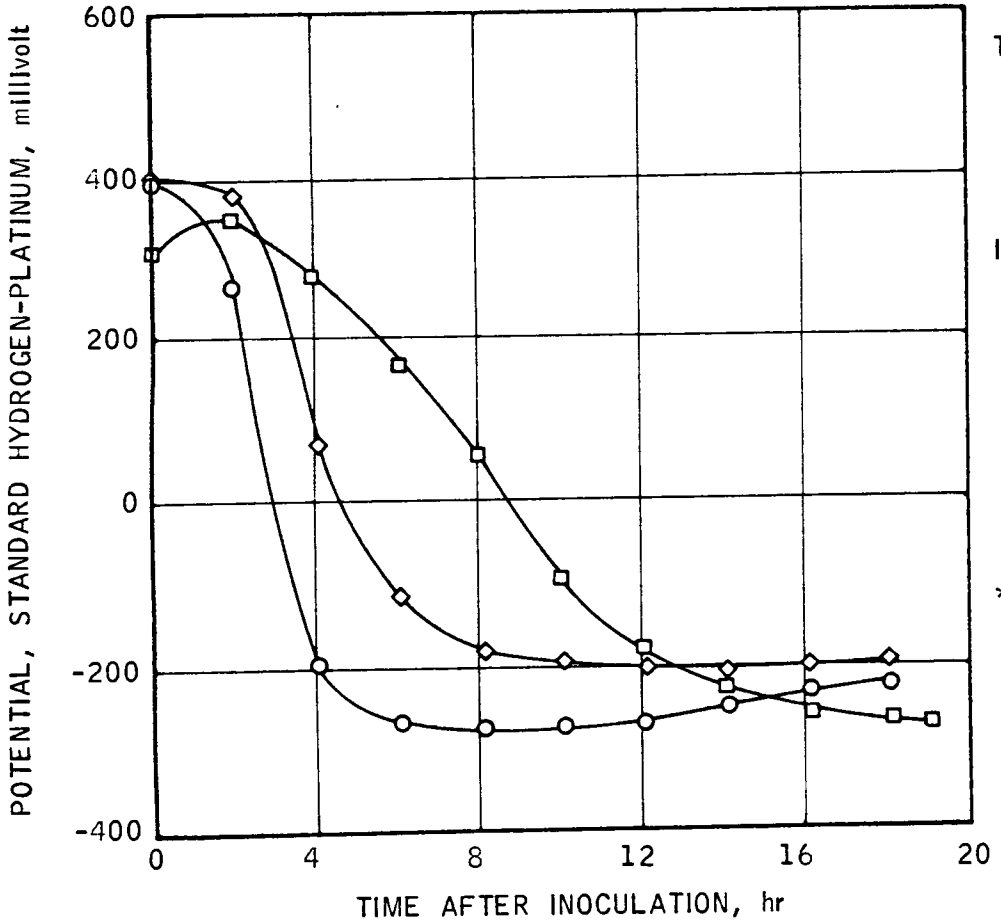
△ = 1:1 (FULL STRENGTH)
○ = 1:4
◁ = 1:16
◇ = 1:32
□ = 1:100
▷ = UNINOCULATED

*1/2 NORMAL CONCENTRATION OF INGREDIENTS (SEE TABLE I)

Figure 42

EFFECT OF CONCENTRATION OF E. COLI ON POTENTIAL CHANGE PRODUCED IN SYNTHETIC MEDIUM C* WITH 1% GLUCOSE

INOCULUM: E. COLI, 18 HR CULTURE GROWN AT 37° C



TEST CONDITIONS:

TEMPERATURE = 24 - 25° C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION

INOCULUM SIZE:

□ = 10^9 ORGANISMS/31 ML
◇ = 2 x 10^9 ORGANISMS/31 ML
○ = 4 x 10^9 ORGANISMS/31 ML

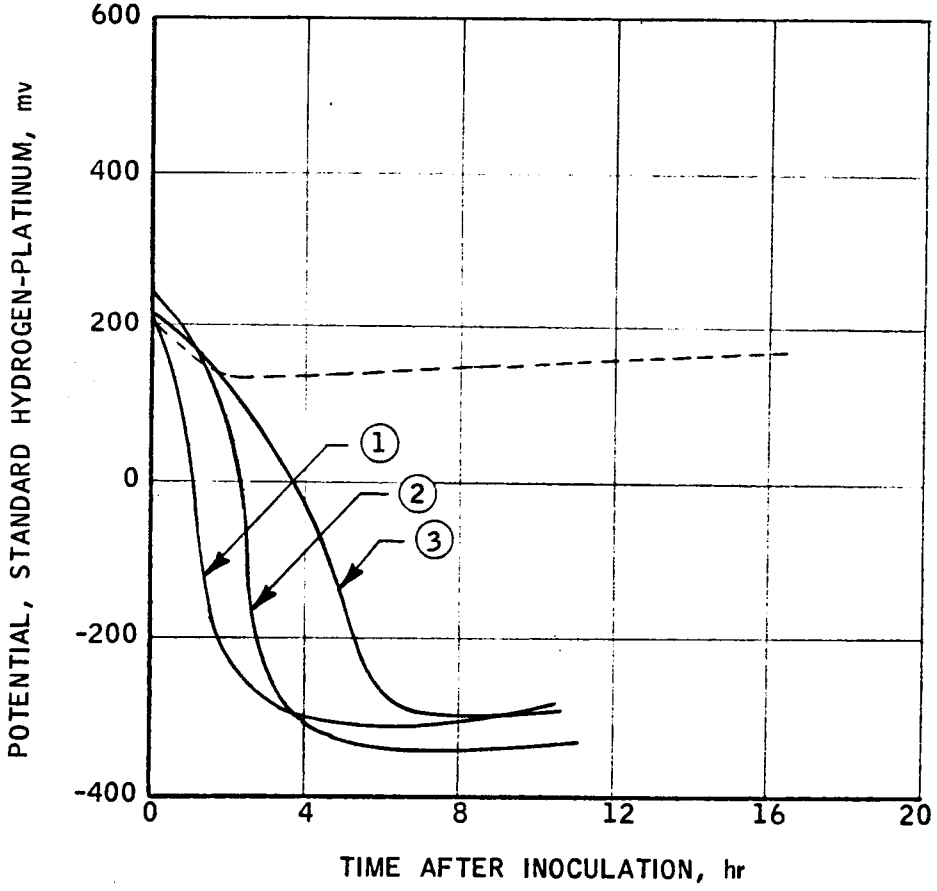
*DESCRIBED IN TABLE I

Figure 43

POTENTIAL CHANGES PRODUCED IN SYNTHETIC MEDIUM C* BY FOURFOLD DILUTIONS OF E. COLI CONCENTRATE

EXPERIMENT NO. 40

INOCULUM: 5 hr CULTURE GROWN AT 37° C FROM MASSIVE INOCULUM
INOCULUM SIZE: CONCENTRATE APPROX. 10^{10} ORGANISMS IN 31 ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
NON-STERILE

----- UNINOCULATED

——— INOCULATED

APPROX. INOCULUM SIZE (DILUTION)

① = 10^{10} ORGANISMS/31ml (1:1)
② = 3×10^{9} (1:4)
③ = 6×10^{8} (1:16)

*DESCRIBED IN TABLE I

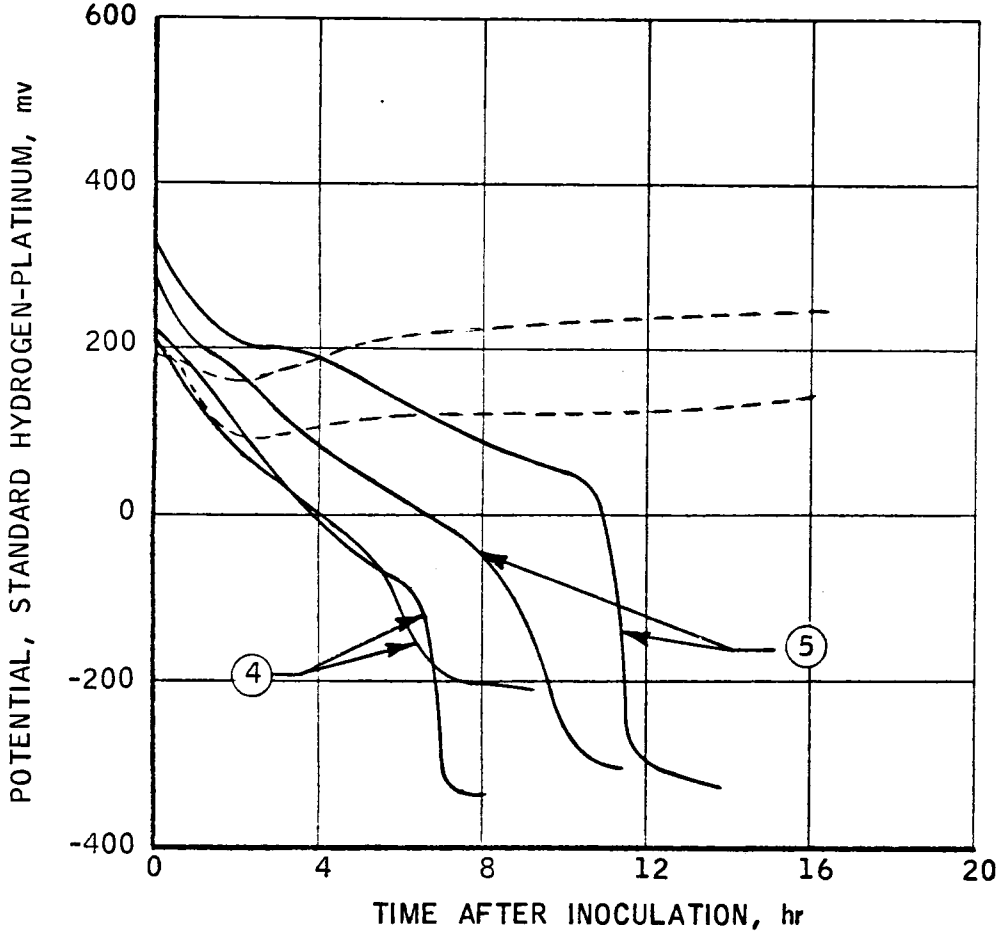
Figure 44

POTENTIAL CHANGES PRODUCED IN SYNTHETIC MEDIUM C* BY FOURFOLD DILUTIONS OF E. COLI CONCENTRATE

EXPERIMENT NO. 40

INOCULUM: 5 hr CULTURE GROWN AT 37°C FROM MASSIVE INOCULUM

INOCULUM SIZE: CONCENTRATE APPROX. 10^{10} ORGANISMS IN 31 ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
NON-STERILE

- - - - - UNINOCULATED

——— INOCULATED

APPROX. INOCULUM SIZE (DILUTION)

(4) = 2×10^8 (1:64)

(5) = 4×10^7 (1:256)

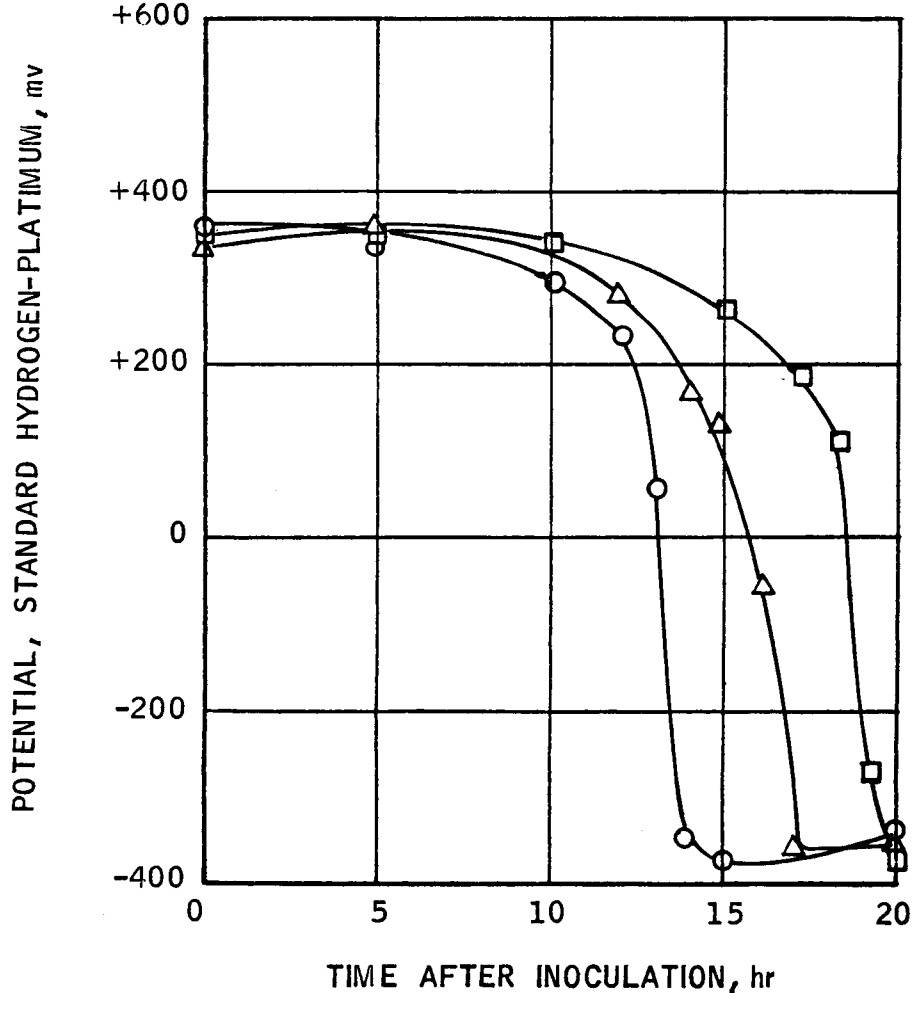
*DESCRIBED IN TABLE I

Figure 45

CHANGES IN POTENTIAL OF TRYPTICASE SOY BROTH* PRODUCED BY DIFFERENT INITIAL CONCENTRATIONS OF ESCHERICHIA COLI

EXPERIMENT NO. 67

INOCULA: 18 hr CULTURE GROWN AT 37°C IN TRYPTICASE SOY BROTH



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

TESTS:

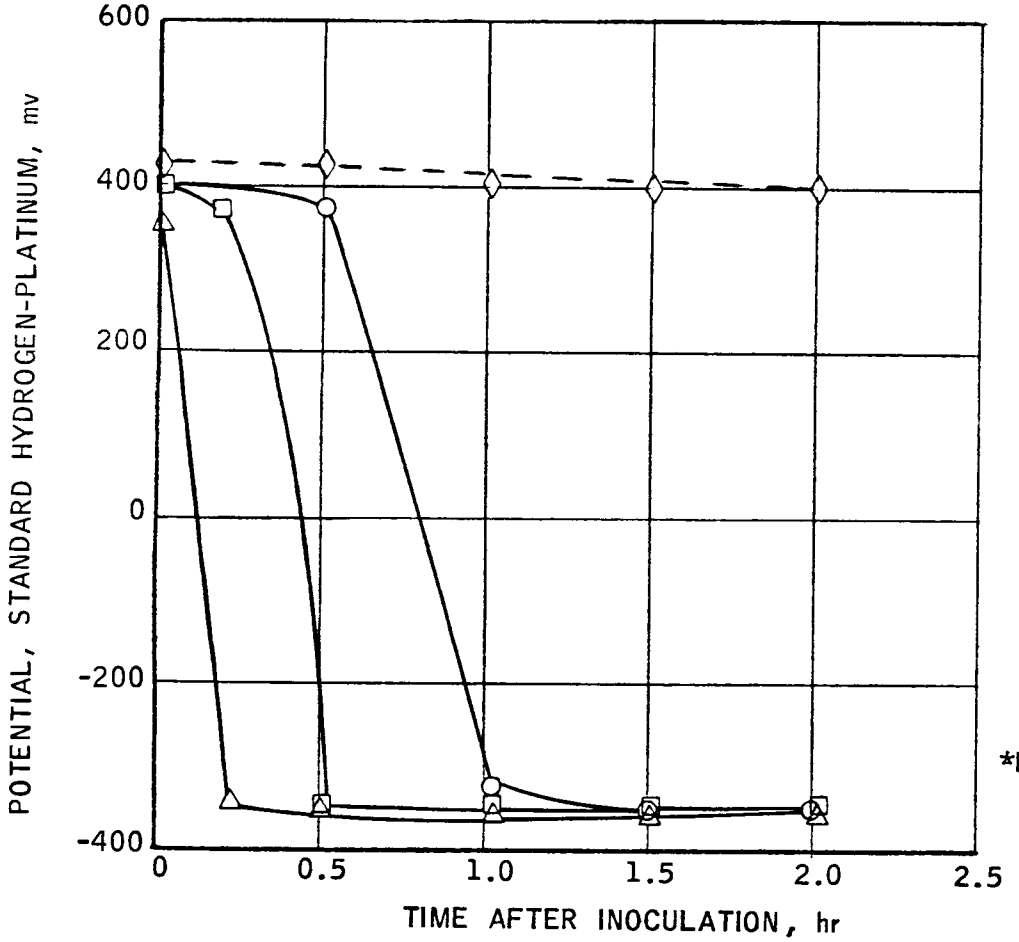
○ = APPROX. 10^4 ORGANISMS/ML
△ = APPROX. 10^3 ORGANISMS/ML
□ = APPROX. 10^2 ORGANISMS/ML

*DESCRIBED IN TABLE I

Figure 46

EFFECT OF TEMPERATURE UPON POTENTIAL CHANGES PRODUCED IN SYNTHETIC MEDIUM C* BY E. COLI

EXPERIMENT NO. 42
INOCULUM: 23 hr CULTURE GROWN AT 37°C
INOCULUM SIZE: APPROX. 2×10^{10} ORGANISMS IN 31 ml



TEST CONDITIONS:

ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
NON-STERILE

- - - - - UNINOCULATED
——— INOCULATED

TEMPERATURE

△ = 35°C ± 2°C
□ = 25°C ± 2°C
○ = 15°C ± 2°C
◇ = AVERAGE OF THREE CONTROLS AT 15, 25, AND 35°C FROM WITHIN A 30 mv RANGE

*DESCRIBED IN TABLE I

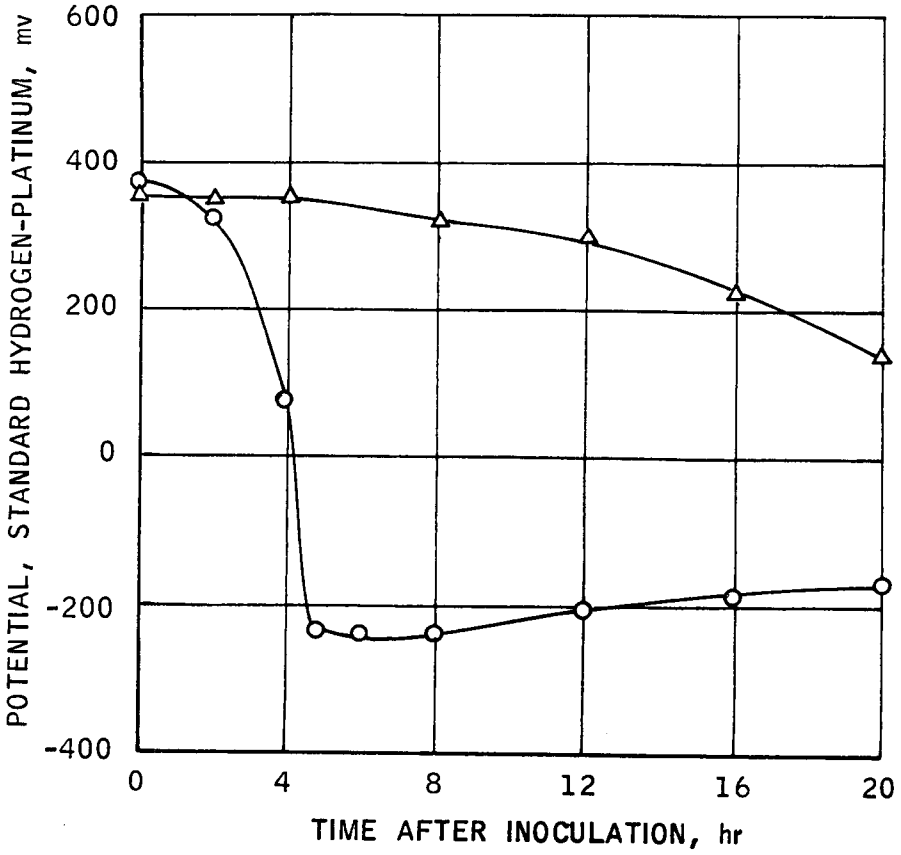
Figure 47

POTENTIAL CHANGE PRODUCED BY E. COLI IN SYNTHETIC MEDIUM C* AND MODIFIED MEDIUM C*

EXPERIMENT NO. 51

INOCULUM: 19 hr CULTURE GROWN AT 37° C

INOCULUM SIZE: APPROX. 10^7 ORGANISMS IN 31 ml



TEST CONDITIONS:

ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

MEDIUM AND TEMPERATURE

△ = SYNTHETIC C, 25°C

○ = MODIFIED C, 37°C

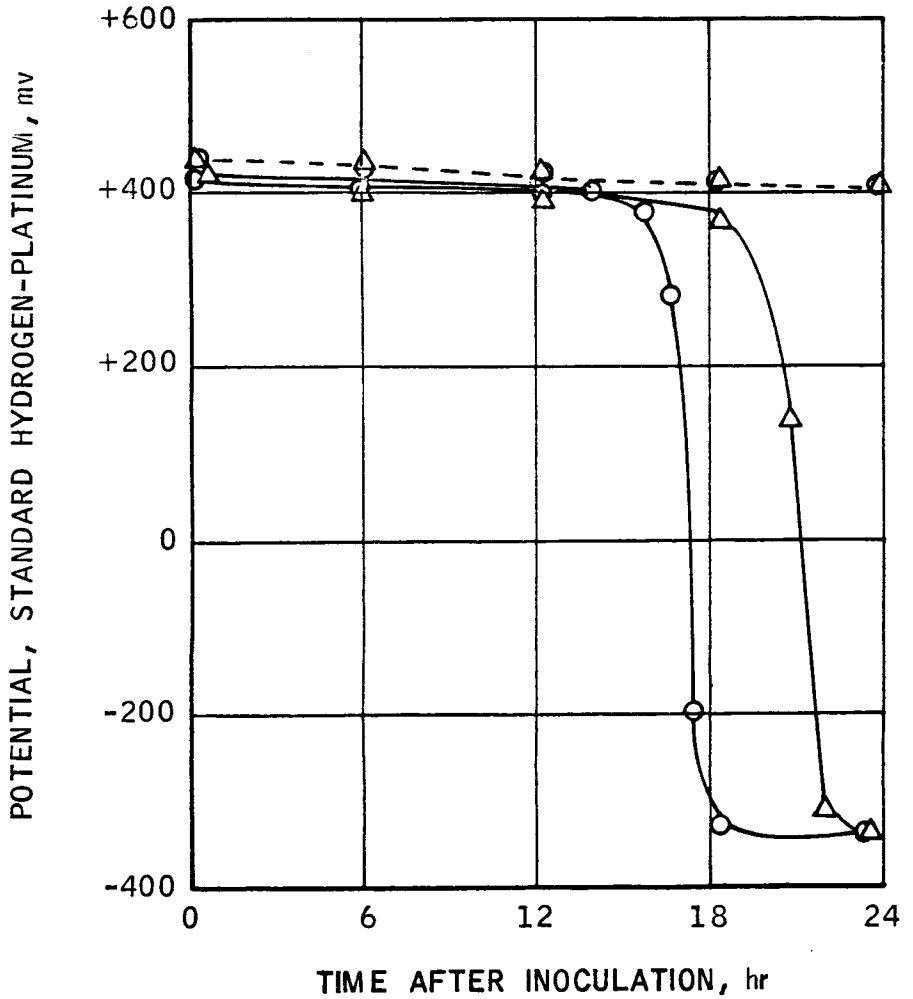
*DESCRIBED IN TABLE I

Figure 48

CHANGES IN POTENTIAL OF SYNTHETIC MEDIUM C* COMPARED TO SYNTHETIC MEDIUM C* IN WHICH MICROBES HAVE PREVIOUSLY GROWN

EXPERIMENT NO. 66

INOCULA: ALL 17 hr CULTURES GROWN AT 37°C IN TRYPTICASE SOY BROTH*
INOCULA SIZE: APPROX. 3×10^5 ORGANISMS/MILLILITER; ESCHERICHIA COLI



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION

ASEPTIC

--------- UNINOCULATED

——————— INOCULATED

TESTS:

Δ = SYNTHETIC MEDIUM C*

O = SYNTHETIC MEDIUM C* IN WHICH E. COLI HAS PREVIOUSLY GROWN

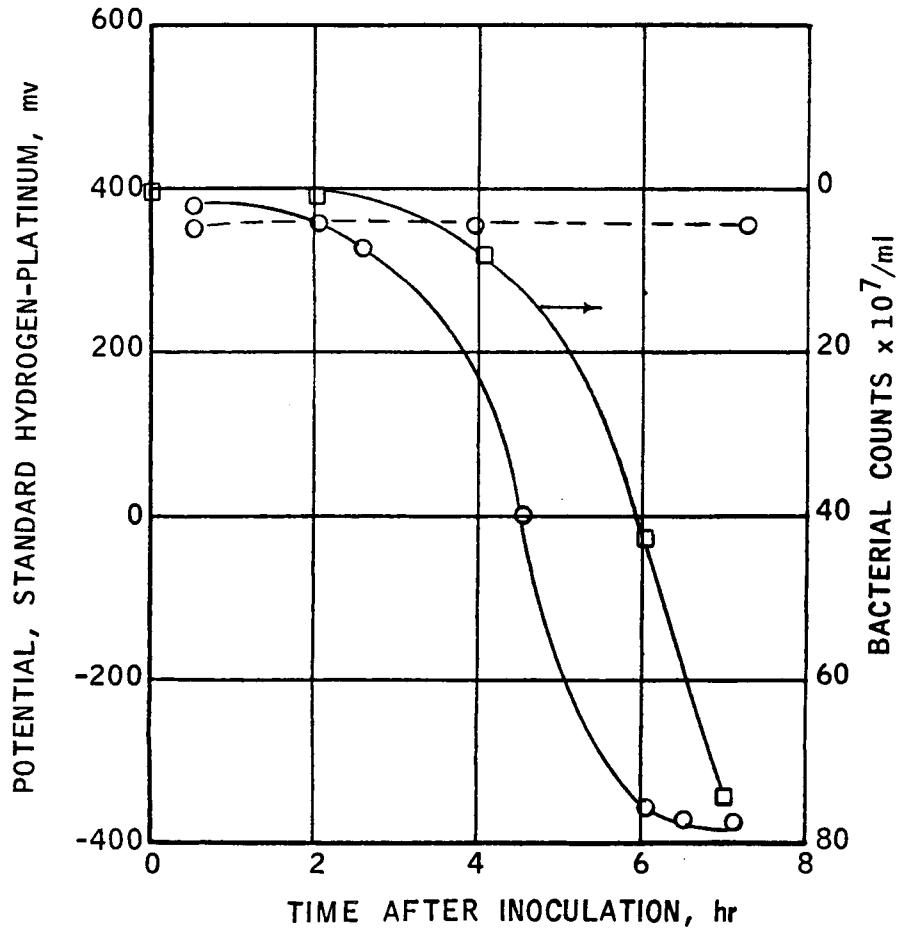
*DESCRIBED IN TABLE I

Figure 49

CHANGES IN POTENTIAL AND VIABLE COUNT BY E. COLI IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 55

INOCULUM: E. COLI, 18 hr CULTURE GROWN AT 37°C



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION
ASEPTIC

- - - - - - UNINOCULATED

——— INOCULATED

TESTS:

O = POTENTIAL

□ = VIABLE COUNT (PLATE)

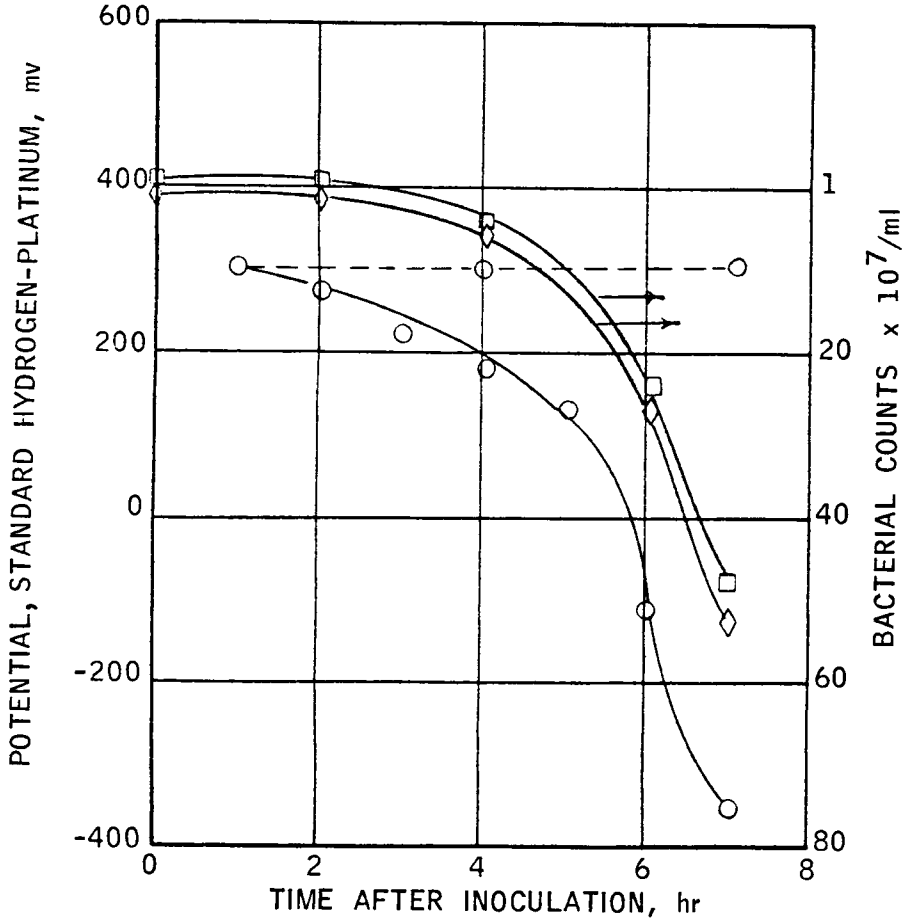
*DESCRIBED IN TABLE I

Figure 50

CHANGES IN POTENTIAL, VIABLE COUNT, AND COULTER COUNT BY E. COLI IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 56

INOCULUM: E. COLI, 18 hr CULTURE GROWN AT 37°C



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION
ASEPTIC

TESTS:

○ = POTENTIAL

□ = COULTER COUNTS

◇ = VIABLE COUNTS

*DESCRIBED IN TABLE I

- - - - UNINOCULATED

——— INOCULATED

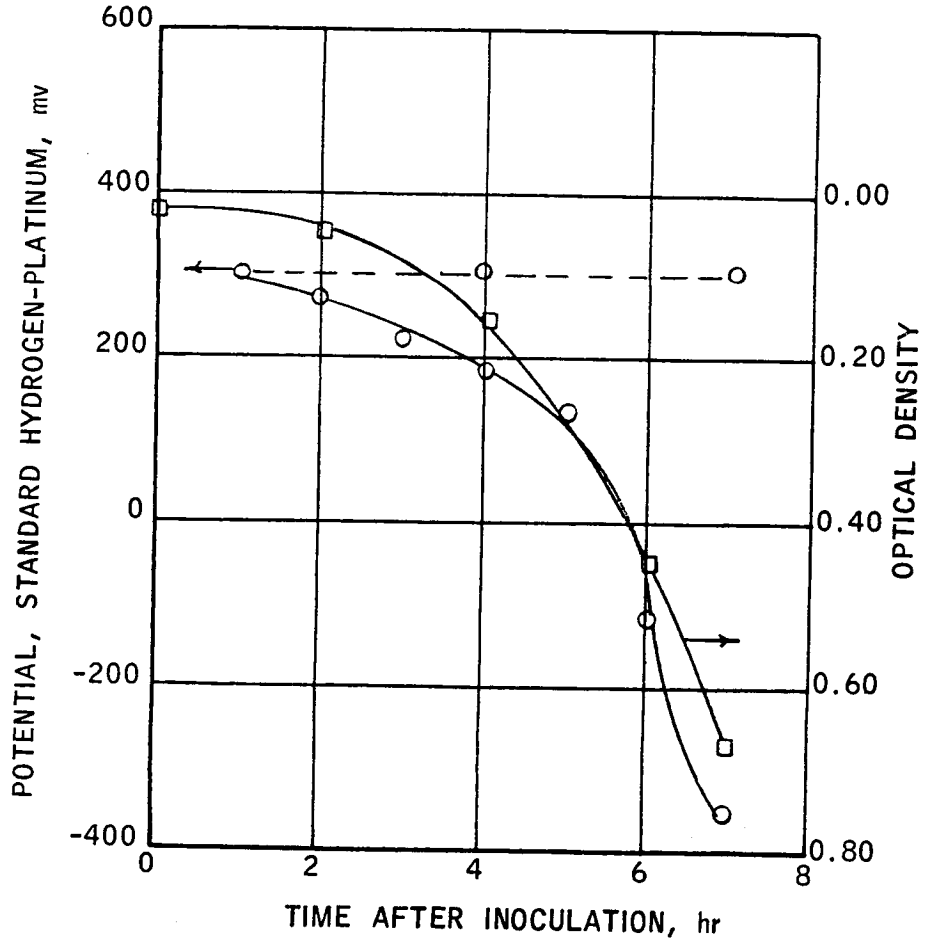
Figure 51

CHANGES IN POTENTIAL AND OPTICAL DENSITY BY E. COLI IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 56

INOCULUM: E. COLI, 18 hr CULTURE GROWN AT 37°C

INOCULUM SIZE: APPROX. 10^7 ORGANISMS/ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION
ASEPTIC

------ UNINOCULATED

—————— INOCULATED

TESTS:

O = POTENTIAL

□ = OPTICAL DENSITY

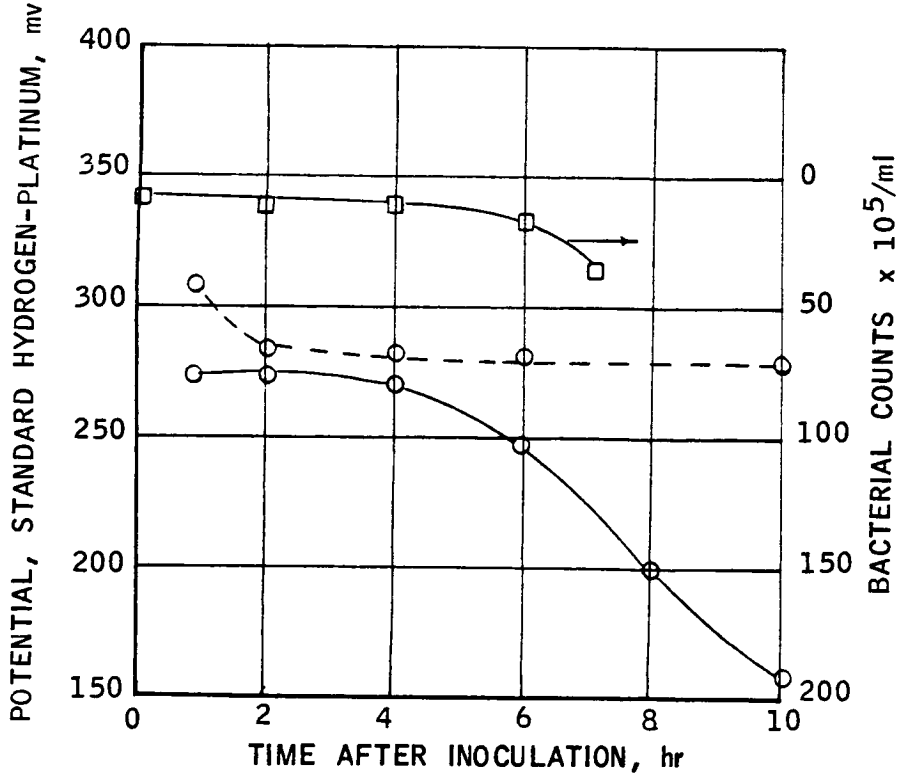
*DESCRIBED IN TABLE I

Figure 52

CHANGES IN POTENTIAL AND VIABLE COUNT BY B. SUBTILIS IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 59

INOCULUM: B. SUBTILIS, 18 hr CULTURE GROWN AT 37°C



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION
ASEPTIC

- - - - - - UNINOCULATED

——— INOCULATED

TESTS:

○ = POTENTIAL

□ = VIABLE COUNT

*DESCRIBED IN TABLE I

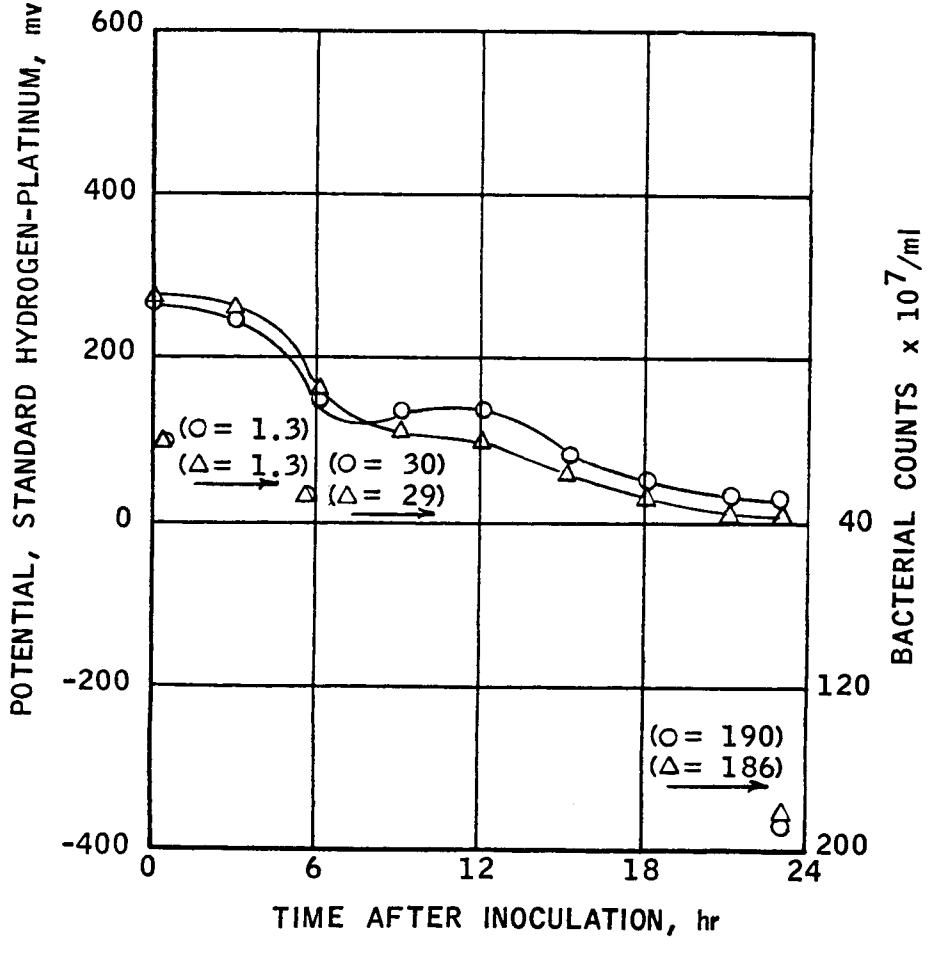
Figure 53

REDOX REPRODUCIBILITY COMPARED TO BACTERIAL COULTER COUNTS

EXPERIMENT NO. 60

MEDIUM: TRYPTICASE SOY BROTH*

INOCULUM: SERRATIA MARCESCENS, 18 hr CULTURE GROWN AT 37°C



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION
ASEPTIC

TESTS:

O = FLASK 24

Δ = FLASK 26

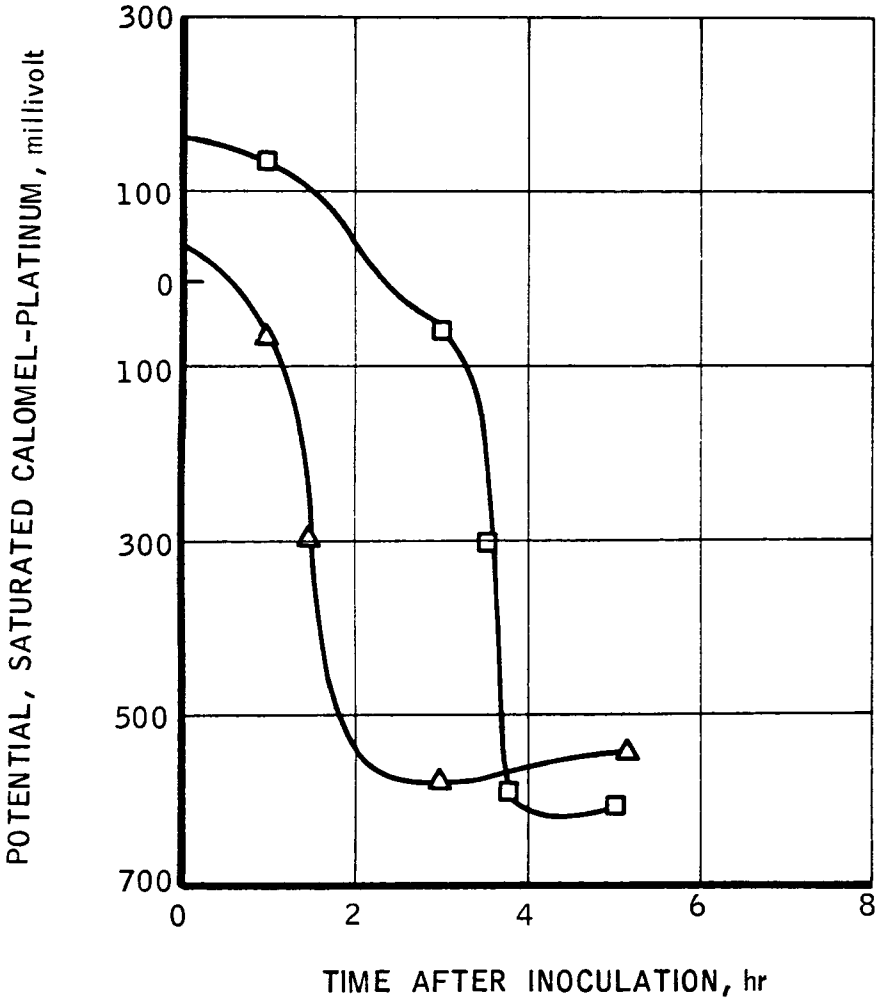
(FLASKS 24 AND 26 WERE DUPLICATE TESTS)

*DESCRIBED IN TABLE I

Figure 54

EFFECT OF GLUCOSE UPON POTENTIAL CHANGE PRODUCED BY E. COLI IN TRYPTICASE SOY BROTH*

INOCULA: E. COLI; GROWN AT 37°C FOR 17-1/3 HOURS
INOCULA SIZE: 2.4×10^7 CELLS/ml



TEST CONDITIONS:

ATMOSPHERE = AMBIENT
CONTINUOUS STIRRING
ASEPTIC

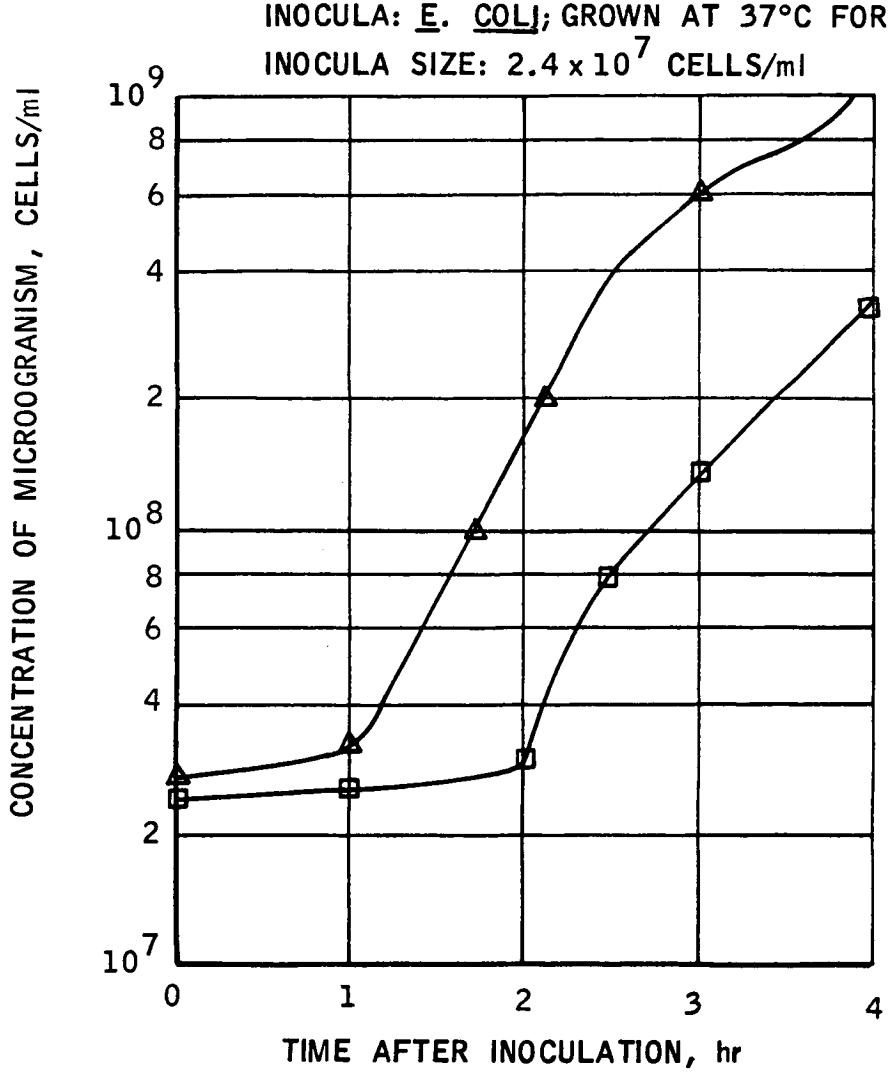
□ = TRYPTICASE SOY BROTH*, 27°C

Δ = TRYPTICASE SOY BROTH + 1% ADDED GLUCOSE, 37°C

DESCRIBED IN TABLE I

Figure 55

EFFECT OF GLUCOSE UPON THE NUMBER OF *E. COLI* GROWN IN TRYPTICASE SOY BROTH*



TEST CONDITIONS:

ATMOSPHERE = AMBIENT
CONTINUOUS STIRRING
ASEPTIC

□ = TRYPTICASE SOY BROTH*, 27°C

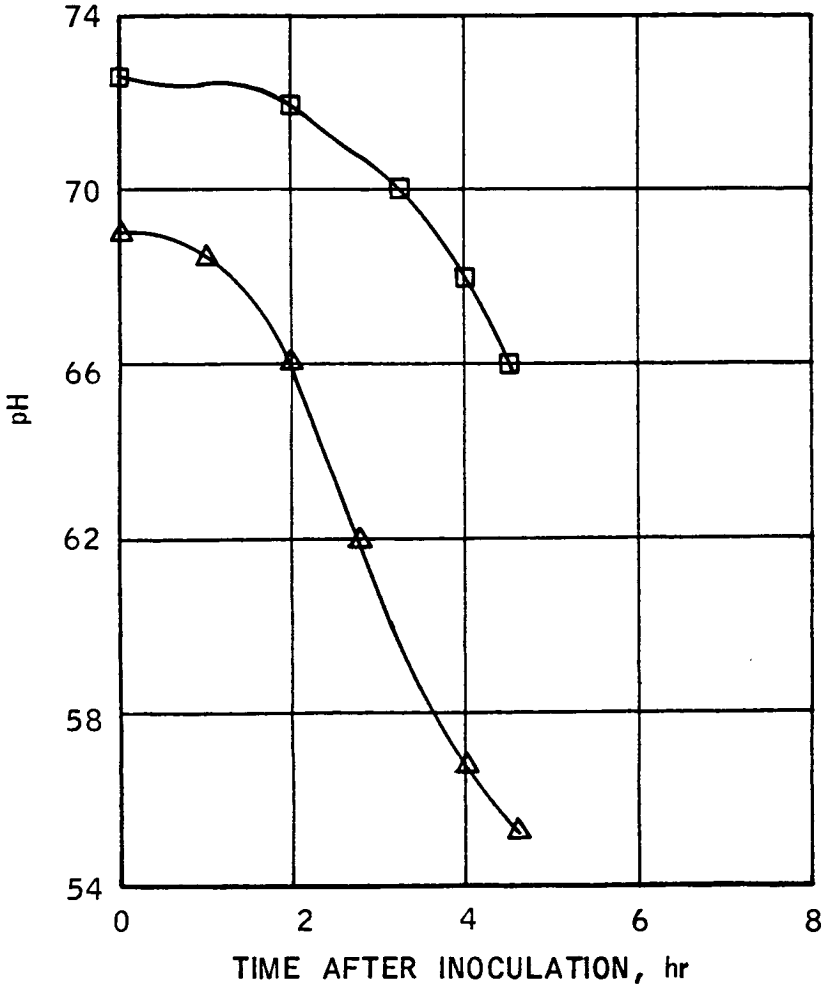
Δ = TRYPTICASE SOY BROTH + 1% ADDED GLUCOSE, 37°C

*DESCRIBED IN TABLE I

Figure 56

EFFECT OF GLUCOSE UPON THE pH CHANGE
PRODUCED BY E. COLI IN TRYPTICASE SOY BROTH*

INOCULA: E. COLI; GROWN AT 37°C FOR 17 1/3 HOURS
INOCULA SIZE: 2.4×10^7 CELLS/ml



TEST CONDITIONS:

ATMOSPHERE = AMBIENT
CONTINUOUS STIRRING
ASEPTIC

□ = TRYPTICASE SOY BROTH*, 27°C

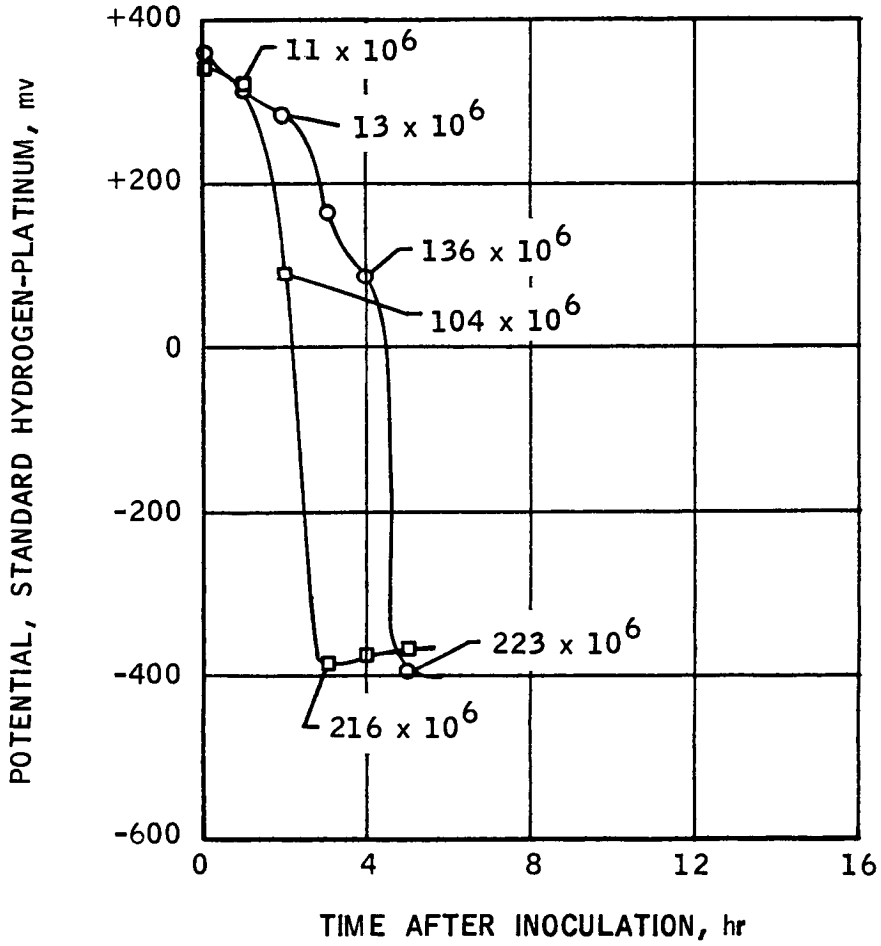
△ = TRYPTICASE SOY BROTH + 1% ADDED GLUCOSE, 37°C

*DESCRIBED IN TABLE I

Figure 57

CHANGES IN POTENTIAL PRODUCED BY E. COLI IN TRYPTICASE SOY BROTH* AT 27°C. AND 37°C.

INOCULA: E. COLI, 18 hr CULTURE GROWN AT 37°C.
INOCULA SIZES: 29°C.: 8.2×10^6; 37°C.: 7.5×10^6/ml



TEST CONDITIONS:

ATMOSPHERE = AMBIENT
CONTINUOUS STIRRING
ASEPTIC

TEMPERATURE:

o = 27°C.
□ = 37°C.

BACTERIAL COUNTS/ml:

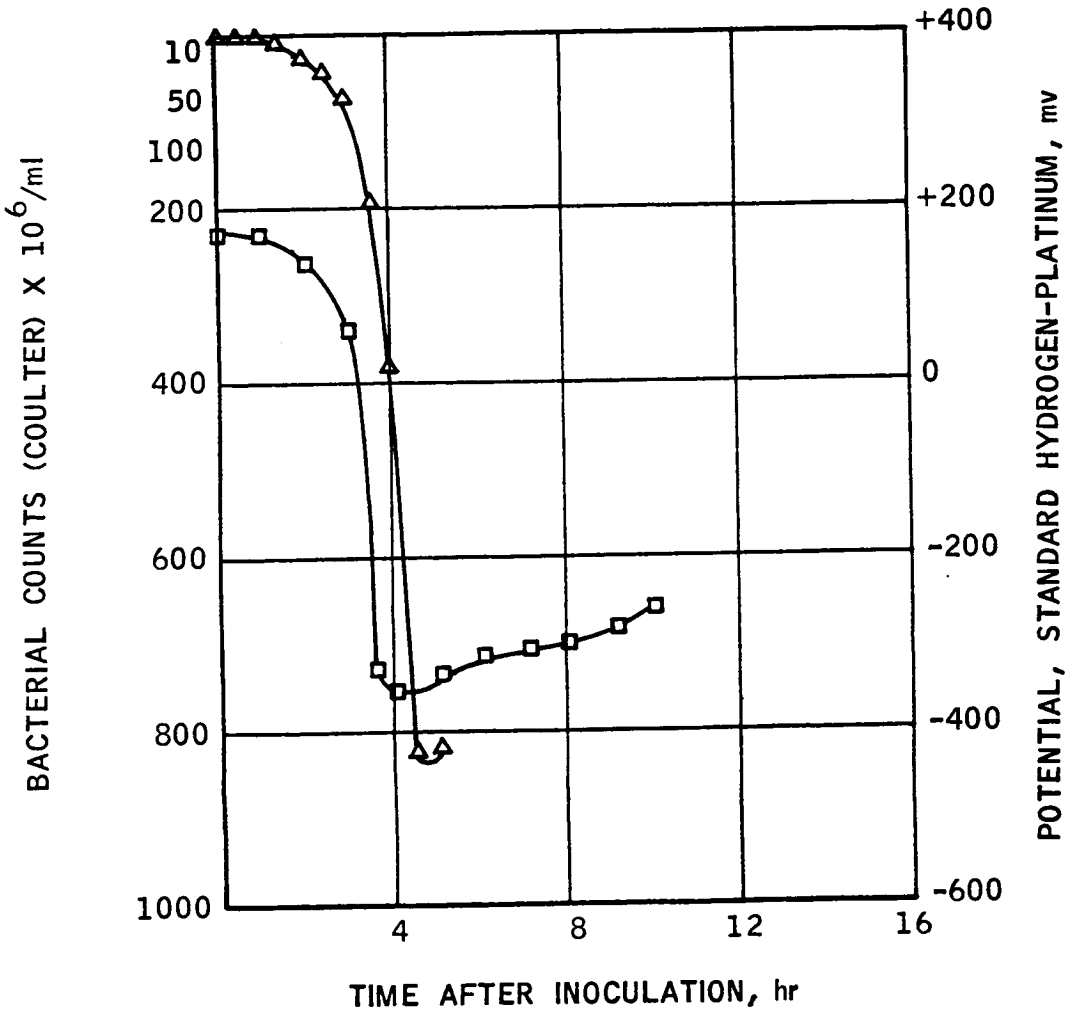
INDICATED ON THE CURVES BY ARROWS

*DESCRIBED IN TABLE I

Figure 58

CHANGES IN POTENTIAL AND COULTER COUNT PRODUCED BY E. COLI IN MODIFIED TRYPTICASE SOY BROTH*

INOCULUM: E. COLI, 17 hr CULTURE GROWN AT 37°C.
INOCULUM SIZE: 3.4×10^6 MICROORGANISMS/ml



TEST CONDITIONS:

TEMPERATURE = 39°C.
ATMOSPHERE = AMBIENT
CONTINUOUS STIRRING
ASEPTIC

CURVES:

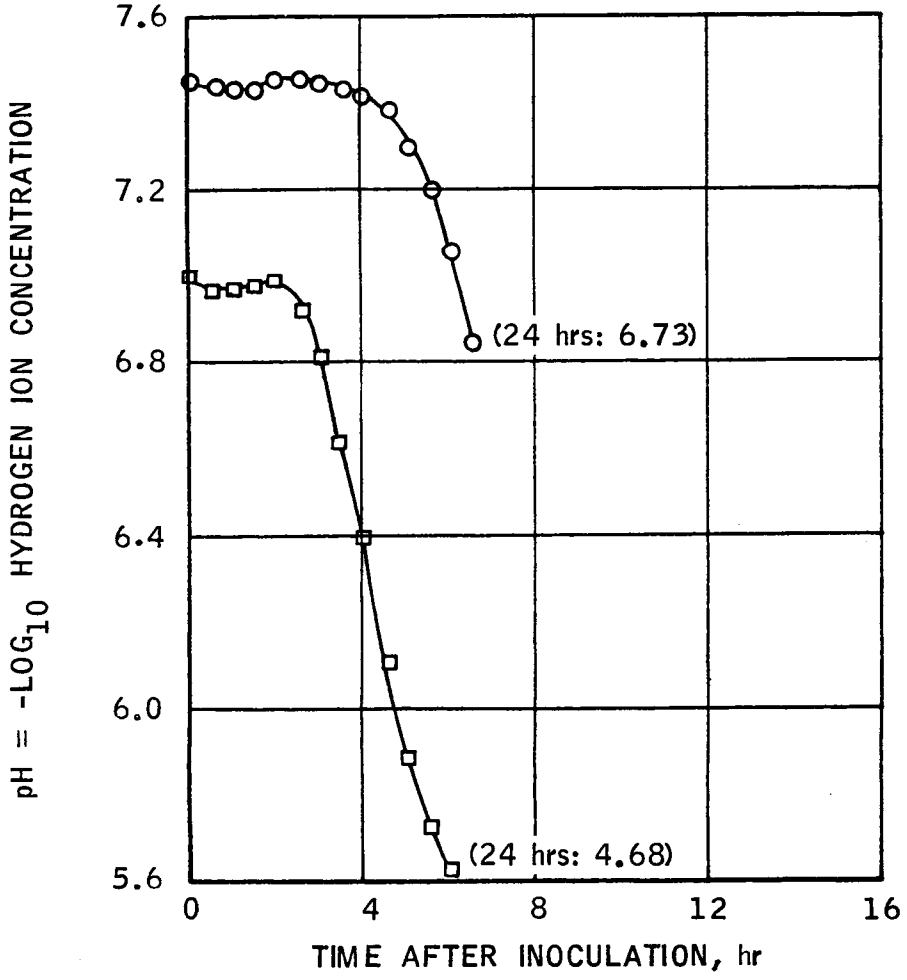
□ = POTENTIAL
△ = COULTER COUNTS

*DESCRIBED IN TABLE I

Figure 59

CHANGES IN pH PRODUCED BY E. COLI IN TRYPTICASE SOY BROTH* AND THE SAME BROTH WITH 1.25% GLUCOSE

INOCULA: E. COLI, 17 hr CULTURE GROWN AT 37°C.
INOCULA SIZES: APPROX. 3×10^6 ORGANISMS/ml



TEST CONDITIONS:

ATMOSPHERE = AMBIENT
CONTINUOUS STIRRING
ASEPTIC

TEST MEDIUM:

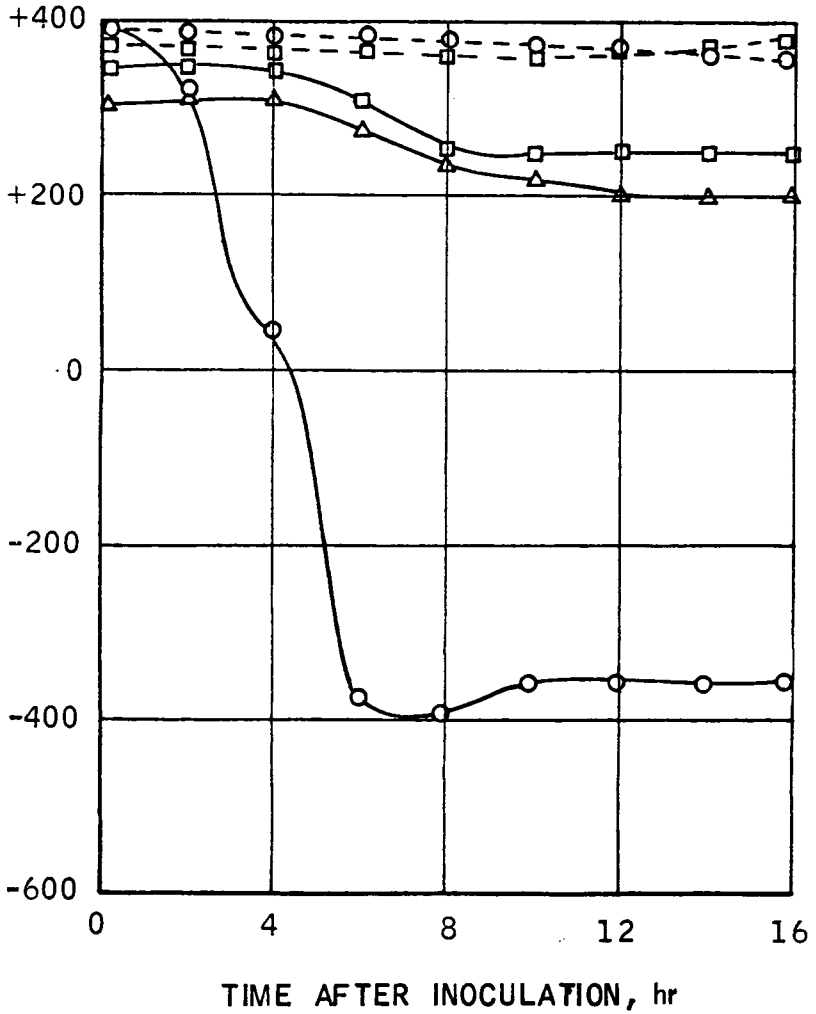
○ = TRYPTICASE SOY BROTH
□ = TRYPTICASE SOY BROTH WITH 1.25% GLUCOSE

*DESCRIBED IN TABLE I

Figure 60

CHANGES IN POTENTIAL PRODUCED BY *E. COLI* IN 0.05 ml AND 31 ml OF TRYPTICASE SOY BROTH*

INOCULA: *E. COLI*, 18 hr CULTURE GROWN AT 37°C.
INOCULA SIZES: APPROX. 10^7 MICROORGANISMS/ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C.
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

– – – – – UNINOCULATED
——— INOCULATED

TEST VOLUME:

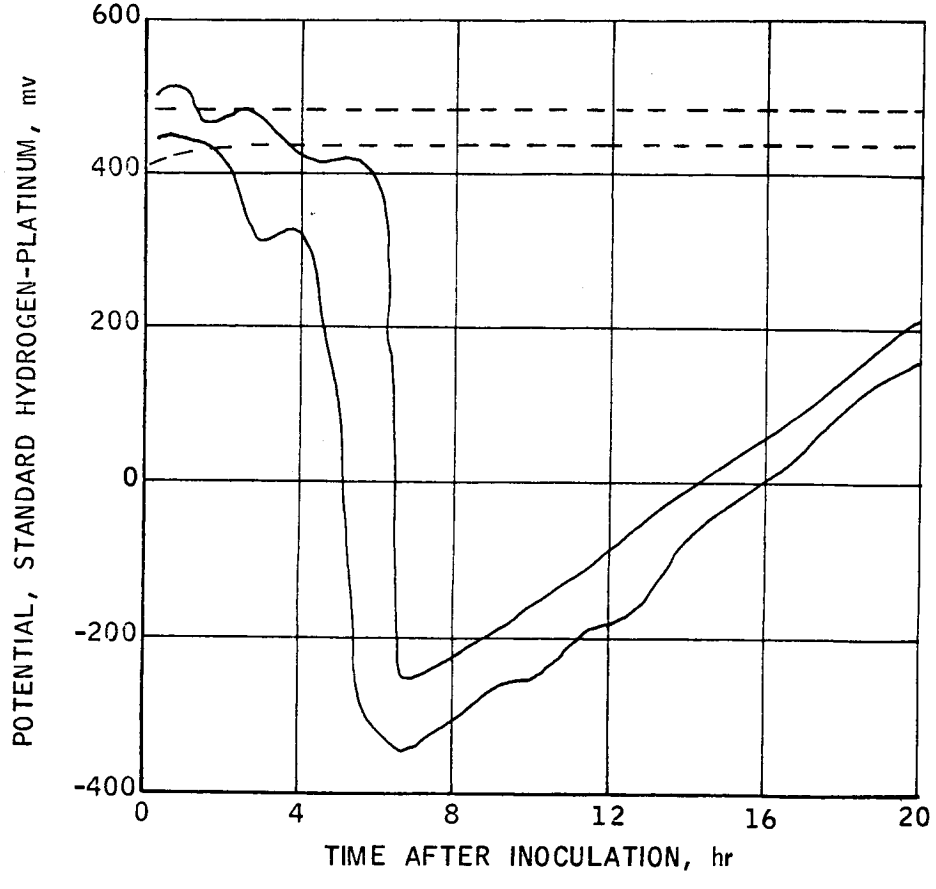
○ = 31 ml
□ = 0.05 ml
△ = 0.05 ml, NITROGEN OVERLAY

*DESCRIBED IN TABLE I

Figure 61

MINIATURIZATION OF MARBAC CELL
(DUPLICATES)

INOCULUM: E. COLI, 22 hr CULTURE GROWN AT 37°C
INOCULUM SIZE: APPROXIMATELY 10^7 MICROORGANISMS / ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT, NO AGITATION AFTER INOCULATION

ASEPTIC

TOTAL VOLUME = 0.4 ml AS COMPARED TO 31 ml FOR REGULAR TEST

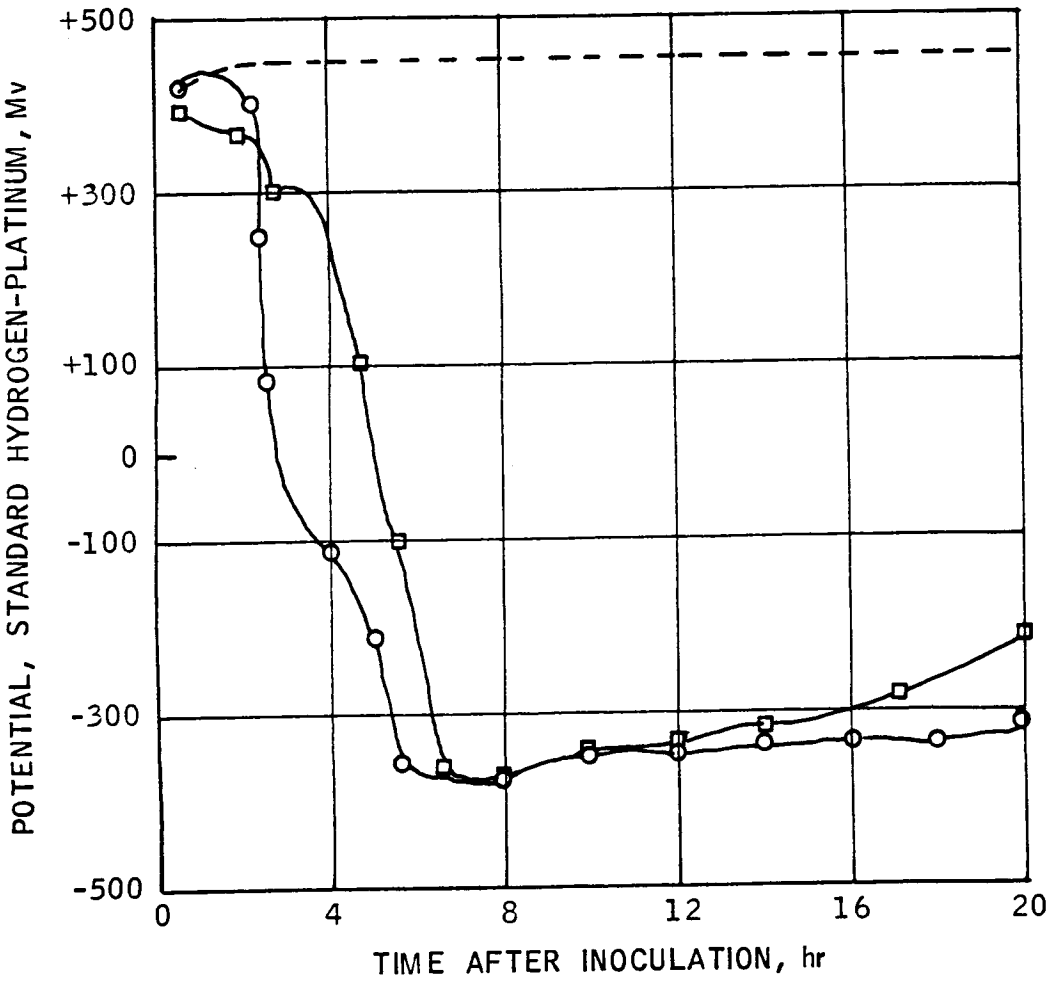
- - - - - UNINOCULATED

——— INOCULATED

Figure 62

MINIATURIZATION OF MARBAC CELL: EFFECT OF NITROGEN BLANKET

INOCULUM: E. COLI, 17 HOUR CULTURE, GROWN AT 37°C
INOCULUM SIZE: APPROXIMATELY 10^7 MICROORGANISMS/MILLILITER
MEDIUM: TRYPTICASE SOY BROTH*



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C
ATMOSPHERE = NITROGEN
ASEPTIC
TOTAL VOLUME = 0.4 ml AS COMPARED TO 31 ml FOR REGULAR TEST

– – – – UNINOCULATED

○ = N_2 OVERLAY PROMPTLY SEALED IN CELL

□ = N_2 OVERLAY NOT SEALED IMMEDIATELY, POSSIBLY PERMITTING ACCESS OF OXYGEN

*DESCRIBED IN TABLE I

Figure 63

TABLE I

MEDIA

NUTRIENT	INGREDIENTS	grams/liter
Nutrient broth*	beef extract	3
	peptone	5
Brain heart infusion*	calf brains, infusion from	200
	beef heart, infusion from	250
	protoese-peptone	10
	dextrose	2
	NaCl	5
	disodium phosphate	2.5
Fluid thioglycollate broth*	casitone	15
	yeast extract	5
	dextrose	5
	sodium chloride	2.5
	l-cystine	0.5
	thioglycollic acid	0.3 (ml)
	agar	0.75
	resazurin	0.001
Synthetic medium C	NH_4Cl	2
	Na_2HPO_4	6
	KH_2PO_4	3
	NaCl	3
	$MgCl_2$	0.01
	Na_2SO_4	0.026
*DIFCO	Glucose	1

TABLE I (Cont'd)

NUTRIENT	INGREDIENTS	grams/liter
Modified synthetic medium C	NH_4Cl	2
	Na_2HPO_4	0.6
	KH_2PO_4	3
	NaCl	3
	$MgCl_2$	0.01
	Na_2SO_4	0.026
	glucose	10
Trypticase soy broth	trypticase	17
	phytone	3
	NaCl	5
	K_2HPO_4	2.5
	glucose	2.5
Modified trypticase soy broth	trypticase	17
	phytone	3
	NaCl	5
	K_2HPO_4	2.5
	glucose	12.5
	yeast extract	0.1
Nitrate broth (Difco)	beef extract	3
	peptone	5
	KNO_3	1

TABLE I (Cont'd)

NUTRIENT	INGREDIENTS	grams/liter
Starkey liquid	peptone	5
	beef extract	3
	yeast extract	0.2
	$MgSO_4 \cdot 7H_2O$	3
	Na_2SO_4	1.5
	Ferrous ammonium sulfate	0.2
	glucose	5
Thiobacillus thioparus media (Source: UCLA)	$Na_2S_2O_3 \cdot 5H_2O$	5
	NH_4Cl	1
	$MgCl_2$	0.5
	KH_2PO_4	0.4
	K_2HPO_4	0.6
	$FeCl_3$	0.020
Basal salt solution of selective media*	$MgSO_4 \cdot 7H_2O$	0.2
	K_2HPO_4	1.0
	$FeSO_4 \cdot 7H_2O$	0.05
	$CaCl_2$	0.02
	$MnCl_2 \cdot 4H_2O$	0.002
	$NaMoO_4 \cdot 2H_2O$	0.001

* (Ref: Stanier, R.Y., D. Doudoroff, and E.A. Adelberg, The Microbial World, Prentice-Hall, Inc., Englewood Cliffs, N.J. (1964), 453)

TABLE II

REPRODUCIBILITY OF POTENTIAL READINGS
OF DYMEC DIGITAL VOLTMETER

Eight consecutive samples taken of each channel, 5 seconds apart. Readings in millivolts. Microbes inoculated 18 hours previously. All media are described in Table I.

1 Second Sampling Period

Synthetic Medium C		Trypticase Soy Broth		Nutrient Broth		Nitrate Broth	
1	2	3	4	5	6	7	8
-626.2	-615.7	-588.9	-599.6	-600.7	-576.3	+52.9	-199.0
-626.2	-615.6	-589.0	-599.6	-600.7	-576.3	+52.8	-197.8
-626.2	-615.6	-589.0	-599.6	-600.7	-576.3	+52.7	-197.4
-626.2	-615.6	-588.9	-599.6	-600.7	-576.3	+52.6	-197.0
-626.2	-615.6	-588.9	-599.6	-600.7	-576.2	+52.5	-195.0
-626.2	-615.6	-588.9	-599.6	-600.7	-576.2	+52.3	-193.7
-626.2	-615.6	-588.9	-599.6	-600.7	-576.2	+52.2	-193.2
-626.1	-615.6	-589.0	-599.6	-600.7	-576.2	+52.0	-192.8

0.1 Second Sampling Period

1	2	3	4	5	6	7	8
-625.5	-616.4	-588.9	-599.8	-601.1	-577.1	+22.4	-296.4
-625.5	-616.4	-589.1	-599.8	-601.0	-577.1	+22.8	-295.6
-625.5	-616.4	-589.0	-599.9	-601.1	-577.1	+23.0	-295.0
-625.6	-616.4	-588.9	-599.8	-601.1	-577.1	+22.9	-294.2
-625.5	-616.4	-589.0	-599.4	-601.1	-577.1	+23.2	-293.5
-625.5	-616.4	-589.0	-599.8	-601.1	-577.1	+23.4	-292.4
-625.6	-616.4	-589.0	-599.8	-601.0	-577.1	+23.9	-291.5
-625.5	-616.4	-589.1	-599.8	-601.0	-577.1	+24.1	-290.2

TABLE III

ELECTRODE PREPARATION

1. Calomel electrodes are filled with saturated potassium chloride solution, with at least one crystal of potassium chloride allowed to remain in the electrode. Saturated potassium chloride solution is either purchased commercially (Beckman Instruments, Inc.) or prepared by adding 400 grams of potassium chloride per liter of distilled water, heating to 40°C or above with stirring, then cooled to room temperature. Excess crystals must be present.

2. Side (filling) arms of calomel electrodes must be closed when not in use to avoid evaporating of potassium chloride solution and drying of electrode fiber. The side arm must be open during use, to allow flow of potassium chloride solution through the fiber.

3. Calomel electrodes are not to be cleaned routinely, other than by rinsing with distilled water, because of danger of contaminating the electrode fiber.

4. Platinum electrodes are disinfected if exposed to pathogens and are routinely cleaned in detergent solution, followed by soft rubbing with a mild abrasive (e.g., baking soda or Bon Ami). The electrodes may then be immersed for one minute in warm nitric acid solution. If this is not adequate, the electrode is cleaned cathodically in dilute hydrochloric acid at a current of 1 milliampere for 1/2 minute.

5. After cleaning and thorough rinsing with distilled water, both electrodes are immersed in 1:1000 mercuric chloride solution for at least one hour, preferably overnight, for sterilization. Just prior to use, the electrodes are rinsed several times with sterile, distilled water.

6. At least one pair of calomel and platinum electrodes are retained for calibration purposes only and are never used for actual tests.

7. A potential difference of less than 25 millivolts between the test and calibration electrodes must be demonstrated prior to starting a formal experiment. If these limits are exceeded, the faulty electrodes are corrected by either cleaning, refilling, or are permanently rejected, as the case may be.

TABLE IV

BACTERIAL COUNTS

Plate Counts

Technician	Average Count 20 Plates		Averages	
	First Reading	Second Reading	Low 4 Plates	High 4 Plates
BH	185	184	165	207
ES	181	182	161	206

Composite Data

Average (20 plate) % Deviation from Mean	Average % Deviation from Mean of 4 low Counts	Average % Deviation from Mean of 4 High Counts
6%	11%	11%

Coulter Counts

(20 Counts of Each Study)

Study	Median	Average % Deviation from Median
A	23,308	3%
B	26,412	4%

Study	Average 4 High Counts	Average % Deviation from Median	Average 4 Low Counts	Average % Deviation from Median
A	23,963	3%	21,754	7%
B	28,717	8%	24,704	6%

TABLE V

SOIL SAMPLES

(SAMPLES OBTAINED FROM DR. ROY CAMERON, JET PROPULSION LABORATORY CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA)

TMC SAMPLE NUMBER	JPL SAMPLE NUMBER	LOCATION & DEPTH	TEXTURE	H_2O CONTENT AT TIME OF COLLECTION %	IRON %	AEROBES, $X10^3$/gm SOIL	FACULTATIVE ANAEROBES, POS. HIGHEST DILUTION/ gm	ALGAE POSITIVE HIGHEST DILUTION/ gm
1	196	Little Lake, California Surface 1 in.	-	-	-	-	-	-
2	51	Thermal, California 1/16-6 in.	Sand	0.29	2.1	42	$>10^4$	10^4
3	76-1	Mojave, California 1/4-6 in.	Sand	1.4	1.4	2700	$>10^4$	10^3
4	10-2	White Mts., 12,400 ft 1 to 6 in.	Loam	6.5	2.7	6700	10^7	10
5	124	Valley of 10,000 Smokes, Alaska Surface 1 in.	Loamy Sand	12.7	5.5	2.9	10^4	10^2
6	125	Valley of 10,000 Smokes, Alaska Surface 1 in.	Clay Loam	15.0	5.6	3.2	$>10^5$	10^4

TABLE V (Cont'd)

SOIL SAMPLES

(SAMPLES OBTAINED FROM DR. ROY CAMERON, JET PROPULSION LABORATORY
CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA)

TMC SAMPLE NUMBER	JPL SAMPLE NUMBER	LOCATION & DEPTH	TEXTURE	H_2O CONTENT AT TIME OF COLLECTION %	IRON %	AEROBES, $X10^3$/gm SOIL	FACULTATIVE ANAEROBES, POS. HIGHEST DILUTION/ gm	ALGAE POSITIVE HIGHEST DILUTION/ gm
9	34	Kau Desert, Hawaii Surface 1/2 in.	Sandy Loam	6.3	7.1	13.2	10^6	$> 10^5$
11	47	Last Chance Mountains, Surface 1/2 in.	-	2.4	-	-	-	-

TABLE VI

POTENTIALS PRODUCED BY CONSTITUENTS OF SYNTHETIC MEDIUM C
(From Experiment No. 43)

CONSTITUENT	CONCENTRATION (g/L)	POTENTIAL (mv)
NH_4Cl	2	+292
Na_2HPO	6	+267
KH_2PO_4	3	+342
NaCL	3	+296
$MgCl_2$	0.01	+316
Na_2SO_4	0.02	+338
Glucose	1	+310
Distilled water		+316
All constituents together, except KH_2PO_4		+270
All constituents together, plus KH_2PO_4		+270
All constituents together, except Na_2HPO_4		+320
All constituents together, plus 1/20th Na_2HPO_4 concentration		+282
All constituents together, plus 1/10th Na_2HPO_4 concentration		+376
All constituents together, plus 3/20ths Na_2HPO_4 concentration		+270

All potential readings were to $\pm$ 5 mv with one pair of electrodes (Saturated Calomel-Platinum). Potentials are referenced above to the Standard Hydrogen Electode.

TABLE VII

SYNERGISTIC EFFECTS OF VARIABLES
UPON RATE OF CHANGE OF POTENTIAL

System: *E. coli* in trypticase soy broth

(°C) Temperature	Strength	Concentration of Microorganisms (cells/milliliter)	0.1% Yeast Extract & 1% Dextrose Added	Total Change of Potential (millivolts)	Time Required (hours)
37	One-half	10^8	Yes	600-650	3/4
27	Full	10^8	No	600-650	1
37	One-half	10^7	Yes	650-700	2-1/4
37	Full	10^7	No	700-	5-1/4

I. INTRODUCTION TO PRINCIPLES OF POLAROGRAPHY

The determination of metabolism and/or growth of various microorganisms has been accomplished using the anodic polarization method as an analytical tool. The principal advantage of the polarographic method of analysis is in the detection of small changes in the quantities of ionizable substances in solutions, such as those that might be consumed or produced as a result of the metabolic reactions of microorganisms in appropriate nutrient media.

Before explaining this method of detecting microorganisms as a result of their metabolism, the theoretical basis for anodic polarography will be presented briefly.

It is known in polarography that at relatively positive potentials, oxidation occurs at the dropping mercury electrode to produce mercurous ions. The anodic dissolution of mercury can occur at more negative potentials, if anions are present in solution which form either stable complexes or insoluble precipitates with mercurous ions, since this mass-action influence shifts the thermodynamic potential of the anodic oxidation of mercury to more negative values. As a result of such influences, anodic oxidation potentials for mercury may possibly be superimposed onto cathodic reduction potentials.

Under these conditions, "mixed" potentials are observed. Limiting diffusion current waves are also of a "mixed" nature due to the occurrence of the aforementioned simultaneous oxidation and reduction electrode processes. These "mixed" polarographic waves cannot be used for quantitative analysis of anions.

However, by avoiding the formation of complexes or of insoluble compounds, truly anodic polarization waves may be obtained. Many anions are suitable for this purpose.

It is thus possible to develop single or composite anodic polarization waves with the dropping mercury electrode in the positive potential region (versus the saturated calomel reference electrode), and to use these polarographic waves as a basis for determining the concentration of a particular anion (single wave) or a mixture of anions (composite wave). It would be necessary, of course, to resolve composite waves, in order to determine the effects of individual anions.

Various types of chemical compounds are consumed and others are produced during the metabolism or growth of microorganisms. It is possible to use appropriate nutrient solutions containing the proper combinations of anions (or in some cases, absence of anions initially), in which the incremental concentration changes can be determined by the above-mentioned polarographic method.

It has been shown that various microorganisms can be detected using the anodic polarization method, as a result of their metabolic activities. The method appears to be applicable to the possible detection of microorganisms in Martian soils. A discussion of the preliminary developments in this polarographic method is presented in the following sections.

II. DISCUSSION OF EXPERIMENTAL APPARATUS AND TECHNIQUES

A. Equipment and Apparatus

1. Polarographic Equipment

The polarograph employed in this investigation is a Model E261 Polarecord (Metrohm AG, Herisau, Switzerland). Strip chart recorded polarograms were obtained using appropriate sensitivities and voltage rate scans. Mercury was dislodged into controlled droplets from the capillary by the impulse method built into the Polarecord. All polarographic analyses were run under the same mercury reservoir height (pressure) and with the same capillary so that all analytical results are directly comparable. Since only relative concentrations of anion species were determined, absolute calibration of the dropping mercury electrode was not needed.

All nutrient solutions (both before and after addition of microorganisms) were diluted one-hundred fold with 0.1 M potassium nitrate or with distilled water to bring solutions into the proper concentration range for the anodic polarization analyses. Deoxygenation was accomplished by bubbling dry nitrogen gas through the solutions for ten minutes before each polarographic analysis. Triple-distilled mercury was used in all experiments.

The polarographic equipment and accessories used in this investigation, and the apparatus used in the polarographic soil experiment, are shown in Figures 1 and 2. Typical polarograms obtained in the investigation are shown in Figures 3 and 4. Figure 3 indicates the nature of composite polarographic waves obtained as a result of Escherichia coli growth, and consumption of anions in brain heart infusion media. Similar polarograms are shown in Figure 4 for composite polarographic anodic waves obtained with a typical soil.

2. Materials

Reagent grade inorganic chemicals were used in making up the solutions. Chemicals used and their concentrations are given in the footnotes of the various tables of data. Distilled water was used in all dilutions. Sterilization of all materials in solution was accomplished by autoclaving before the addition of the microorganisms.

The commercial nutrients, and the preparations of nutrient solutions, were described previously (see Table I in the Oxidation-Reduction Potential Section). The soil samples were also described previously (see Table V in the Oxidation-Reduction Potential Section).

B. Bacteriological Aspects of Polarographic Studies

1. Preparation of Cultures

For the polarographic experiments, Escherichia coli was grown in brain heart infusion and in trypticase soy broth for eighteen (18) hours at 37°C. A portion of the cultures (10 milliliters) was centrifuged at 8,000 revolutions per minute for 10 minutes, washed with saline solution, and re-centrifuged. The centrifuged and washed culture was added to 600 milliliters of the medium; this provided a mixture containing 10^7 cells per milliliter.

Samples of the appropriate media were diluted 1:100 with 0.1M potassium nitrate solution for polarographic analysis and pH measurement. Polarographic analyses were obtained at various time intervals after inoculation, with the final reading 21 hours after inoculation; in addition to polarographic analysis and pH measurement, bacterial counts were made of the inoculated media at the same time intervals, by using the Coulter counter (described in Section II.A of the Oxidation-Reduction discussion). The results of the experiment are presented in Table I, where data are included concerning the bacterial counts, pH, and oxidation-reduction potential. The polarographic analyses and counts were made immediately after samples were taken, except that the 5-hour sample was counted after 21 hours, and the pH of all samples was read after 48 hours. Results showed that the oxidation-reduction potential of the inoculated trypticase soy broth changed immediately, while that of the inoculated brain heart infusion began to change after 1-1/3 hours.

The pH of the inoculated brain heart infusion changed from 7.38 to 6.37 after 3-½ hours; it then increased to 6.45 after 5 hours, and to 7.14 after 21 hours. The pH of the inoculated trypticase soy broth decreased from 7.33 to 5.77 after 2½ hours, then increased to 5.83 after 3½ hours and to 6.88 after the 21-hour period.

2. Effect of Concentration of Phosphate Ion on Growth of Escherichia coli: In employing the polarographic technique for the study of the metabolism of microorganisms, it is desirable that changes in concentrations of the electroactive ions be sufficiently large that they can be determined quantitatively. Therefore, one of the phases of the investigation involved the determination of the effect of the concentration of one inorganic species (e.g., phosphate ion) in nutrient broth on the growth of Escherichia coli. Escherichia coli was chosen for this study because the results obtained in the polarographic investigations could be correlated with the vast amount of data already obtained with this organism during this contract. Nutrient broth was chosen as the growth medium because it has a low concentration of inorganic ions, and there was little interference or overlap of other ions in the determination of phosphate ion. The composition of nutrient broth is described in Table I of the Oxidation-Reduction Division.

The Escherichia coli were grown at 37°C (standard incubation temperature) for 28 hours. Phosphate ion was added to the nutrient broth in the form of Na_2HPO_4 and NaH_2PO_4. The range of concentration of salts containing phosphate ion was varied from zero to 13.1 grams per liter; in this range, there was apparently no effect of phosphate ion concentration on the growth of this organism. The pH of the solutions varied with phosphate ion concentration, as shown in Table II.

C. Polarographic Analyses

1. Consumption of Phosphate Ion by Escherichia coli in Nutrient Broth: Escherichia coli was grown in trypticase soy broth (composition is described in Table I of the Oxidation-Reduction Division) at 37°C for 21¼ hours.

Portions of this culture (2.5 milliliters) were then inoculated into 500 milliliters of nutrient broth (described in Table I of the Oxidation-Reduction Division). Duplicate flasks of nutrient broth were inoculated, as well as duplicate flasks containing 6.549 grams of phosphate salts per liter of solution in nutrient broth. The phosphate ion was supplied by equal molarities of Na_2HPO_4 and NaH_2PO_4.

Portions of these samples were then diluted one-hundred-fold with 0.1M potassium nitrate or with distilled deionized water, and polarograms were obtained. The polarograms were obtained under three sets of polarographic settings of sensitivity, starting voltage, voltage range, voltage and chart speeds, and with or without impulse to dislodge mercury droplets from the capillary.

2. Consumption of Liquid Starkey Medium by Desulfovibrio desulfuricans: Desulfovibrio desulfuricans was grown anaerobically in liquid Starkey's medium (described in Table I of the Oxidation-Reduction Division) at room temperature, then analyzed polarographically after 4½ and 18½ hours. The polarographic technique was the same as that described above, and polarograms were obtained at various sensitivities, voltage ranges, and voltage and chart speeds.

Desulfovibrio desulfuricans is known to reduce sulfates, and it is a microorganism which has been studied extensively and about which much is known and is available in the literature. Therefore, it was a logical choice of microorganism to be used in establishing the applicability of the polarographic technique of analysis to the present investigations.

Liquid Starkey medium was chosen because it contains only complex organic ingredients and sulfate salts, and there was little interference with the sulfate analysis.

3. Consumption of Media by Thiobacillus thioparus: Thiobacillus thioparus was grown in a special medium (described in Table I of the Oxidation-Reduction Division)for three days at room temperature, with shaking and aeration. The pH was adjusted twice daily with sodium bicarbonate solution. The cells were centrifuged, washed twice with sterile, deionized water, and resuspended (3 milliliters of cell suspension in 27 milliliters of sterile, deionized water).

Thiobacillus thioparus is a sulfide oxidizer ($S_2O_3^{-2}$ to SO_4^{-2}). The organisms were grown in a medium whose formula was obtained with the culture. A washed suspension was placed in the Thiobacillus medium described in Table I of the Oxidation-Reduction Division; another in a solution of $Na_2S_2O_3$ (7 grams per liter of water); a third in a solution of Na_2SO_3 (7 grams per liter of water); and a fourth in sterile, distilled, deionized water. The various media provided a comparison of the effects of the sulfur formed by the microorganisms as they grow; there was the possibility that the microorganisms would metabolize the sulfur compounds without having to grow.

III. RESULTS

The various bacteria-nutrient systems studied polarographically are listed in Table III. This listing of experiments is provided for quick reference in cataloging the nature of the bacterial-nutrient systems. A variety of microorganisms and nutrients was studied in this preliminary investigation as a means of quickly surveying the potentialities of the anodic polarization method for detecting the effects of metabolism.

The results of the polarographic analyses for all the bacterial-nutrient systems studied to date are tabulated in Tables IV through XI. Nutrient concentrations and conditions for the polarographic analyses are shown in the footnotes accompanying each table. Half-wave potentials in the tables refer to an empirically-determined composite anodic wave in each case. It was not possible at this preliminary stage in the development to resolve polarization waves into the components due to individual anion metabolites.

The relative diffusion current change observed with time is an indirect measure of bacterial action. Changes in compositions of solutions, calculated from diffusion current degradations, correspond to the influence of various microorganisms. It should not be inferred, however, that the percentage decrement calculated from the diffusion current changes indicates quantitatively the extent to which microbial action has occurred.

The growth of Escherichia coli at 37°C was studied polarographically in several media (see Tables IV and V). It was found (Table IV) that growth after 4½ hours in brain heart infusion medium was not sufficient to detect a change in anionic metabolites via the polarographic method. After 19 hours, however, diminution of anodic polarization waves corresponded to a consumption of anion metabolite in brain heart infusion.

It was also found (Table VA) that after 18 hours, diminution of the anodic polarization wave corresponded to a somewhat larger percentage consumption of anion metabolite in nutrient broth.

The much higher consumption of anion metabolite evidenced in the nutrient broth experiment for essentially the same elapsed time (18-19 hours) does not necessarily mean that Escherichia coli grew better in nutrient broth than in brain heart infusion. Nutrient broth is reported to contain only organic ingredients. Presumably, the anodic wave observed in this case is due to unknown anions present at small impurity levels. Accordingly, the relatively large percentage change observed in the anodic wave after 18 hours might well be reflecting only a change in anionic impurities, because the anion constituents are present in very small quantities initially (i.e., the impurity level). The effects of such impurities would have to be established in further studies.

At this point in the development, it can be concluded only that the anodic polarization method can be used to detect Escherichia coli growth after 18-19 hours in either of the media studied.

The importance of the right combination of nutrient ingredients is shown by considering the results given in Table VB. The addition of phosphate ions to ordinary nutrient broth is seen to prevent or obscure the previously observed diminution in the composite impurity anodic wave. The fact that the relative diffusion current (wave height) is the same with and without Escherichia coli (polarogrammed both in potassium nitrate and in water) indicates that phosphate ions were not appreciably consumed by Escherichia coli during the 18-hour incubation period at 37°C.

The results given in Table VI indicate that the anodic polarographic method may be capable of better limits of detectability in the system Desulfovibrio desulfuricans - liquid Starkey. After only 5 hours at room temperature, a large change in the anodic polarization wave height was observed. It is not known at this time which anion metabolite (or combination) was diminished during the 5-hour growth period. The medium contains considerable sulfate ions, which are known to be reduced during the growth of Desulfovibrio desulfuricans. It is not known whether the large change observed in the anodic wave corresponds to a utilization of sulfate ions (corresponding to rapid growth of Desulfovibrio desulfuricans) or to the consumption of impurities. Minor quantities of impurities present and consumed even at modest rates could yield large changes in relative wave heights (as discussed previously).

The metabolism of Thiobacillus thioparus in three different sulfur-containing media was also studied. Sodium thiosulfate ($Na_2S_2O_3$) and sodium sulfite (Na_2SO_3) are apparently equally good metabolites for Thiobacillus thioparus (see Tables VIIA and VIIB). The metabolic rate was sufficient to show the consumption of sulfur anion constituents in a 3-hour period, because the organisms had been previously concentrated.

An especially prepared Thiobacillus medium was also used, which is described in Table I of the Oxidation-Reduction Division. Results obtained with this sulfur-containing medium seem to indicate an even greater sensitivity than that discussed above. (See Table VIIC)

The progressive growth of an inoculum of Escherichia coli, first in brain heart infusion and then in trypticase soy broth, was studied at 37°C over a 21-hour period. Growth change influences at various times from 0 to 21 hours were followed using the anodic polarization technique, determination of microorganism population by Coulter counts, and pH measurements. The results of these analyses are given in Tables VIII and IX. Similar results were obtained in both brain heart infusion and trypticase soy broth. Coulter Counts indicated that Escherichia coli continued to grow

for about 5 hours. In both media, anodic polarization data (i.e., percentage of change in diffusion current) and pH values showed a reversal in their progressive patterns or trends at the 2.5-hour time period. It would thus seem that the increase in both the pH and anodic polarization wave height (i.e., decrease in percentage change of diffusion current) after 2.5 hours might well be reflecting the same phenomena. However, it should be pointed out that there was a 2-day lapse between taking of samples and pH determination (the samples were refrigerated during this time). At this stage in the development, the reason for this behavior is not known.

Two soils were added to trypticase soy broth and the progressive metabolism and growth of the various indigenous microorganisms was monitored (see Tables X and XI). Oxidation-reduction potential readings were taken simultaneously with the polarographic studies. It may be seen in the Tables that the polarographic method reflected changes in the composition of the nutrient-soil solution very quickly, because of its ability to detect extremely small amounts of certain ions. Additional studies would have to be conducted to determine the portion of the change at each interval of time that was caused by purely chemical changes due to the addition of soil to the solution, and the portion that resulted from metabolic reactions. These effects could be separated by comparing the data obtained with sterile and non-sterile soils. After separating these effects, it would then be possible to determine the length of time necessary to detect metabolic activity in soils by the polarographic method.

IV. CONCLUSIONS

An anodic polarization method using polarographic techniques has been studied for detecting the growth or metabolism of microorganisms. A variety of organisms and nutrients has been employed in developing the appropriate procedure. It appears that this indirect method of detecting bacteria can be applied to microbial systems capable of sustaining either growth or metabolism, if it can detect changes in concentrations of the anionic metabolites in the nutrient.

Application to microbe detection in soils has been demonstrated. It appears that the polarographic method might well have wide application for detecting bacteria as a result of their metabolism or growth.

POLAROGRAPHIC EQUIPMENT AND ACCESSORIES

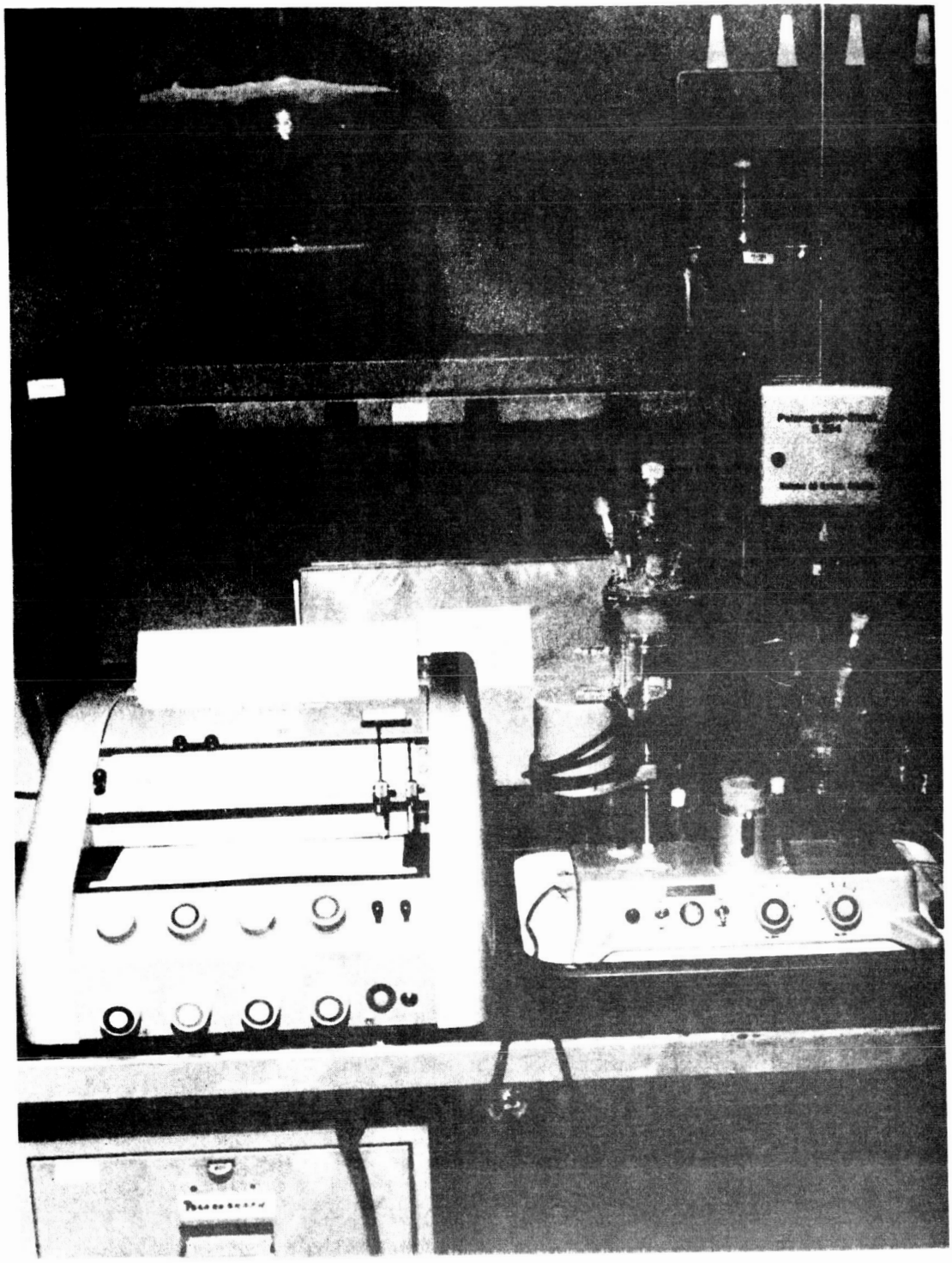


Figure 1

POLAROGRAPHIC SOIL SAMPLE EXPERIMENT

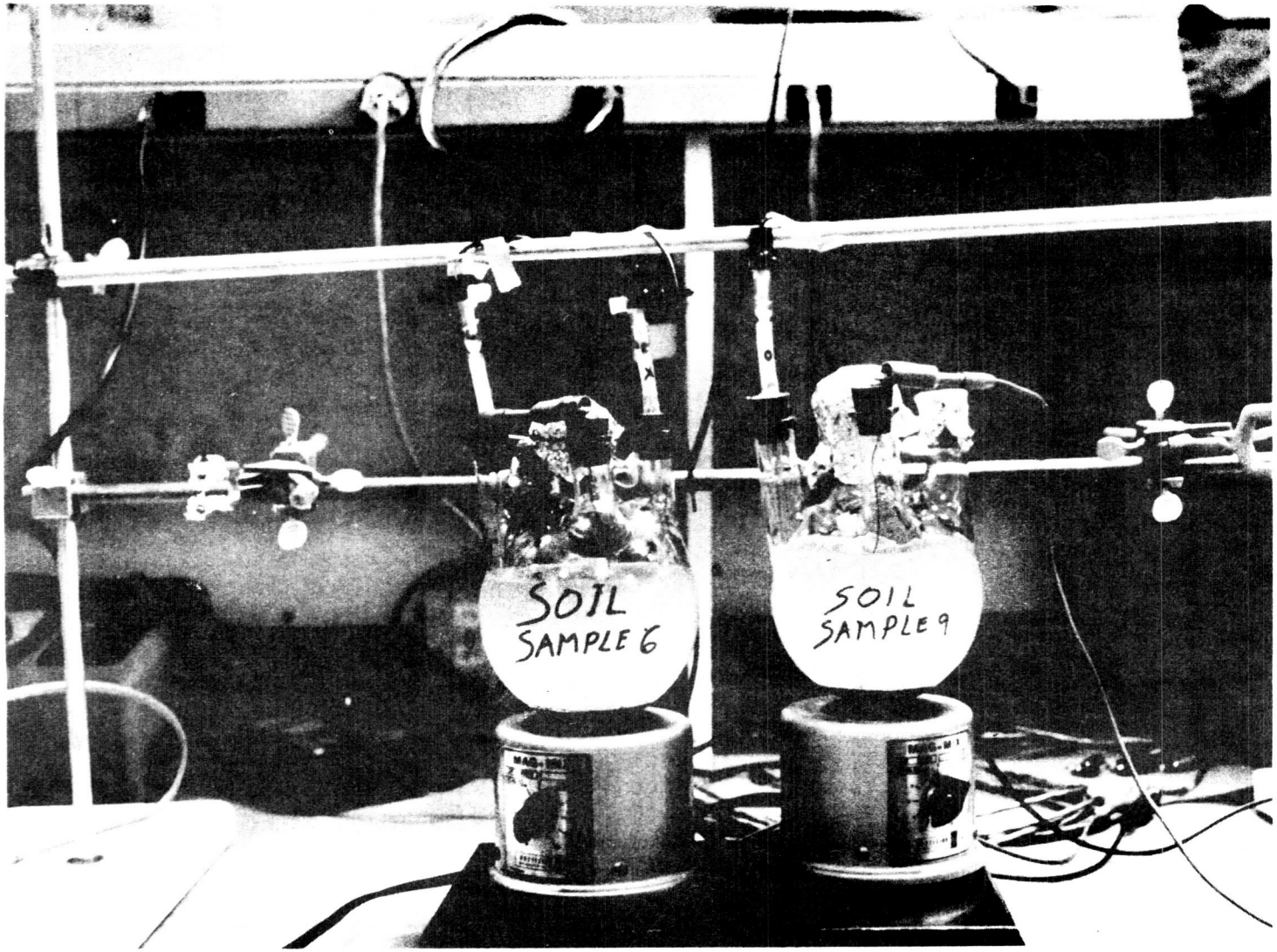


Figure 2

POLAROGRAM OF E. COLI IN BRAIN HEART INFUSION
(BEFORE GROWTH INITIATED)

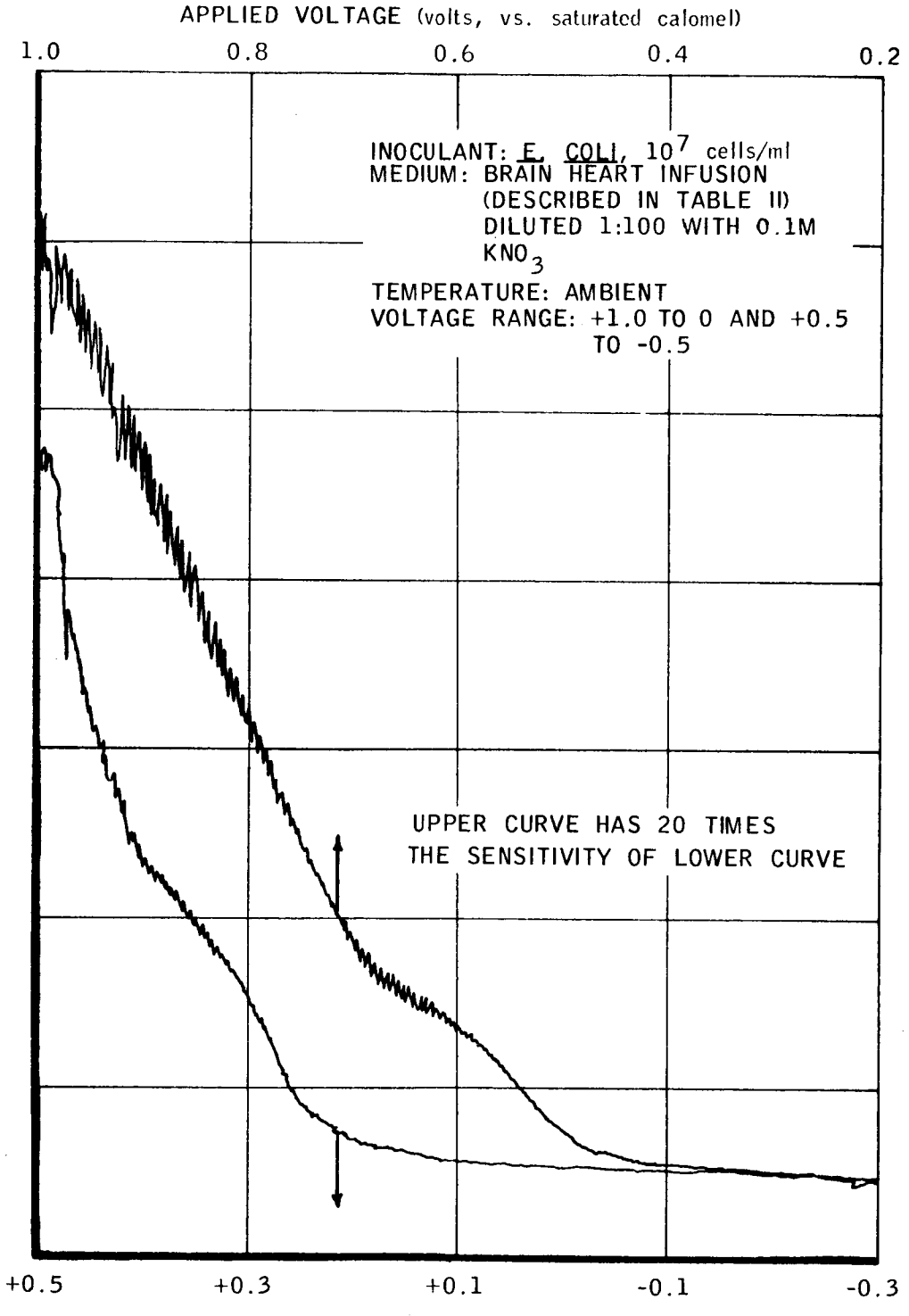


Figure 3

POLAROGRAM OF SOIL SAMPLE IN TRYPTICASE SOY BROTH

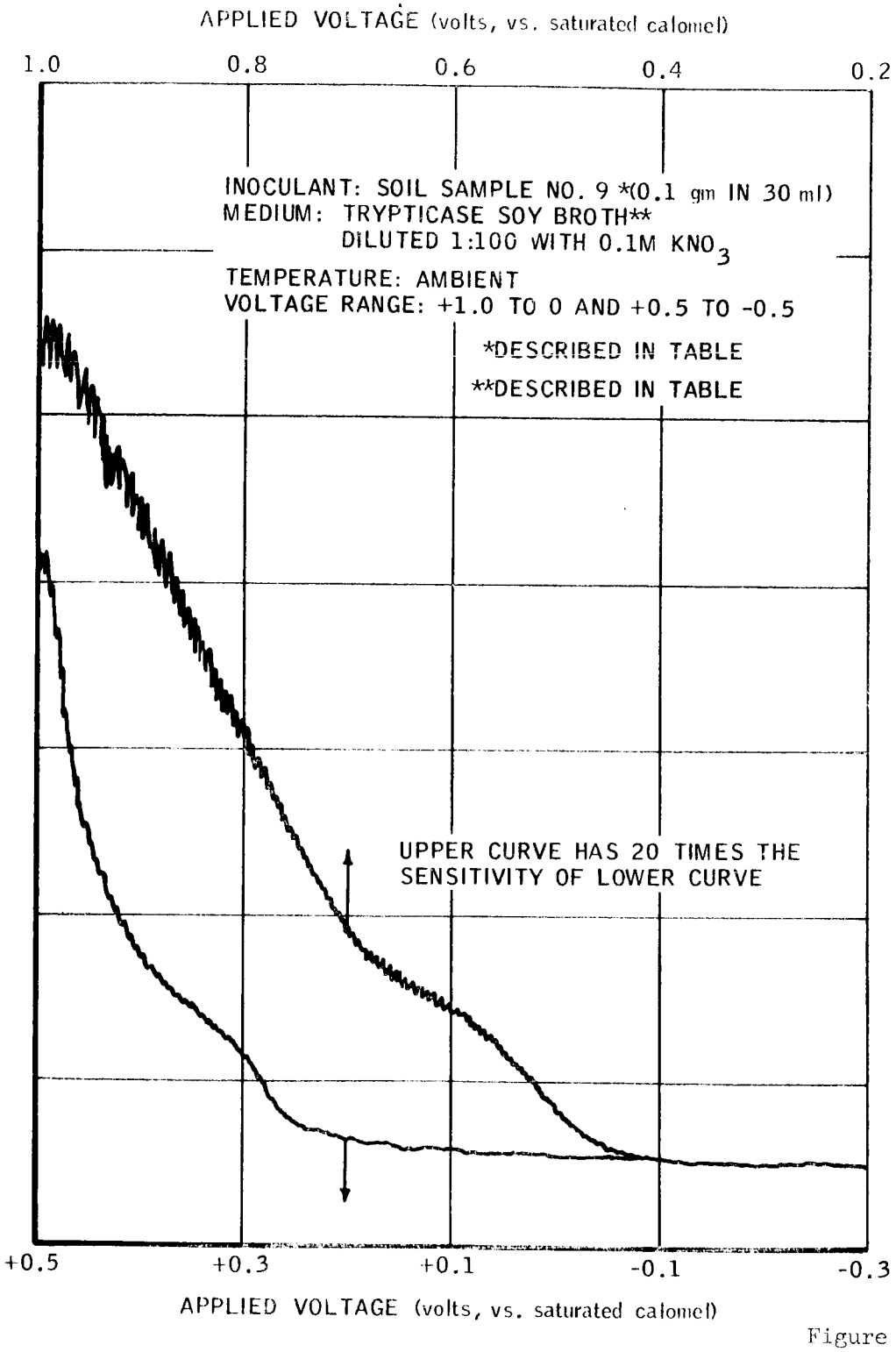
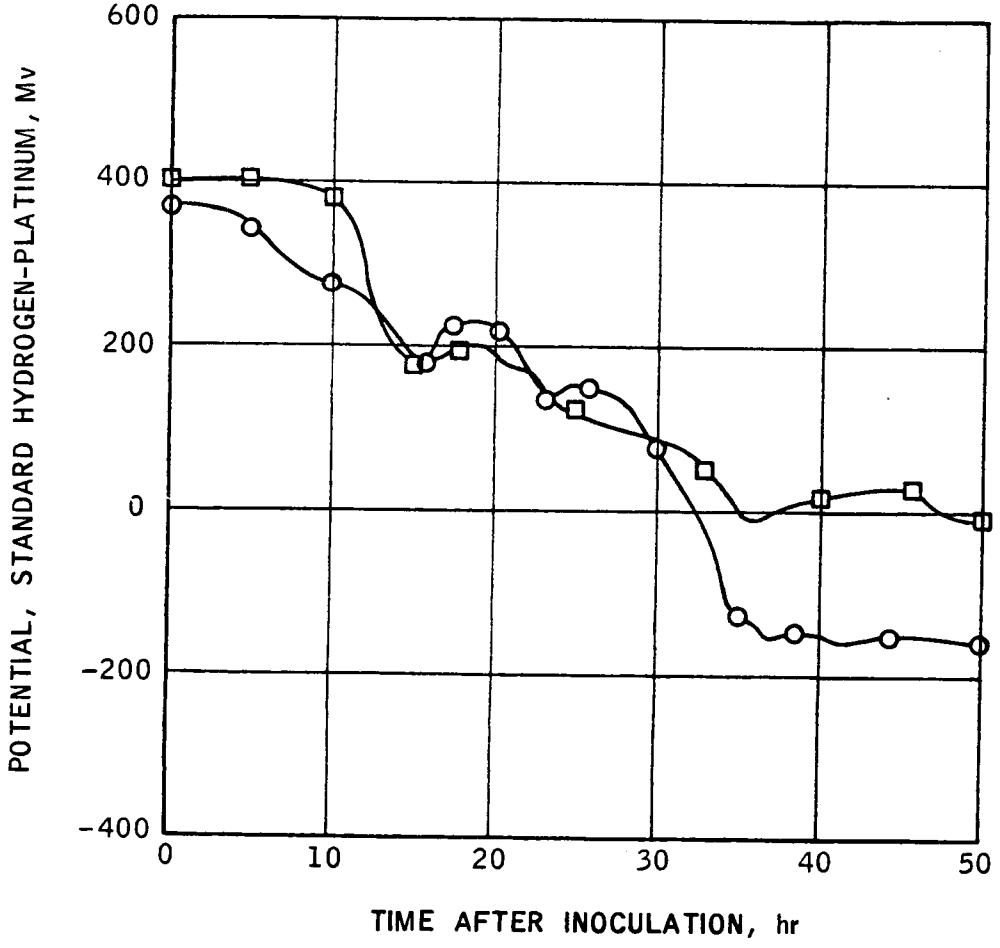


Figure 4

CHANGES IN POTENTIAL PRODUCED BY SOIL SAMPLES* IN TRYPTICASE SOY BROTH**

INOCULUM: 2.83 gm SOIL IN 850 ml MEDIUM



TEST CONDITIONS:

TEMPERATURE = 24-25 °C
ATMOSPHERE = AMBIENT
MAGNETICALLY STIRRED FOR 22-25 min.
ASEPTIC

○ = SOIL SAMPLE 6*
□ = SOIL SAMPLE 9*

* DESCRIBED IN TABLE V
** DESCRIBED IN TABLE I

Figure 5

TABLE I

E. COLI IN MEDIA

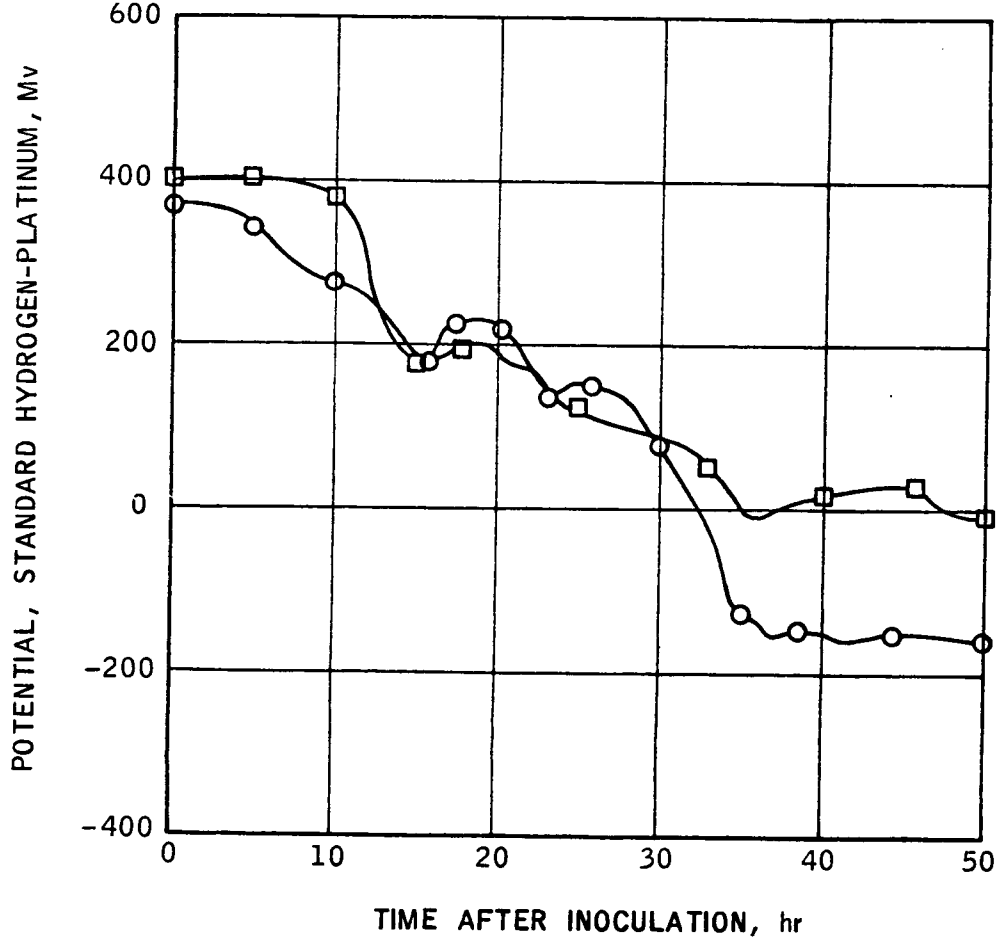
Sample Time (hours after inoculation)	Bacterial Count		pH		Potential (mv.) Saturated Calomel Ref.	
	BHI(1)	TSB(2)	BHI(1)	TSB(2)	BHI(1)	TSB(2)
Uninoculated Blank	-	-	7.38	7.33	+35	+55
0	1.82×10^7	1.36×10^7	7.38	7.31	+35	+55
1/2	1.86×10^7	2.24×10^7	7.38	7.17	+20	-30
1-1/2	3.63×10^7	2.13×10^8	7.33	6.37	-15	-225
2-1/2	1.55×10^8	4.13×10^8	6.70	5.77	-210	-625
3-1/2	1.47×10^9	1.20×10^9	6.37	5.83	-627	-580
5	3.27×10^9	3.23×10^9	6.45	6.00	-605	-580
21	3.33×10^9	3.75×10^9	7.14	6.88	-	-

(1) BHI = brain heart infusion, described in Table

(2) TSB = trypticase soy broth, described in Table

CHANGES IN POTENTIAL PRODUCED BY SOIL SAMPLES* IN TRYPTICASE SOY BROTH**

INOCULUM: 2.83 gm SOIL IN 850 ml MEDIUM



TEST CONDITIONS:

TEMPERATURE = 24-25 °C
ATMOSPHERE = AMBIENT
MAGNETICALLY STIRRED
FOR 22-25 min.
ASEPTIC

○ = SOIL SAMPLE 6*
□ = SOIL SAMPLE 9*

* DESCRIBED IN TABLE V
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Figure 5

TABLE I

E. COLI IN MEDIA

Sample Time (hours after inoculation)	Bacterial Count		pH		Potential (mv.) Saturated Calomel Ref.	
	BHI(1)	TSB(2)	BHI(1)	TSB(2)	BHI(1)	TSB(2)
Uninoculated Blank	-	-	7.38	7.33	+35	+55
0	1.82×10^7	1.36×10^7	7.38	7.31	+35	+55
1/2	1.86×10^7	2.24×10^7	7.38	7.17	+20	-30
1-1/2	3.63×10^7	2.13×10^8	7.33	6.37	-15	-225
2-1/2	1.55×10^8	4.13×10^8	6.70	5.77	-210	-625
3-1/2	1.47×10^9	1.20×10^9	6.37	5.83	-627	-580
5	3.27×10^9	3.23×10^9	6.45	6.00	-605	-580
21	3.33×10^9	3.75×10^9	7.14	6.88	-	-

(1) BHI = brain heart infusion, described in Table

(2) TSB = trypticase soy broth, described in Table

TABLE II

VARIATION OF pH WITH CONCENTRATION OF PHOSPHATE ION IN NUTRIENT BROTH

Phosphate ion was supplied by equal molarities of Na_2HPO_4 and NaH_2PO_4; original concentration 13.0975 gms. per liter of solution, other concentrations obtained by dilution.

Total Weight of Phosphate Salts (gms./liter solution)	pH
13.0975	6.77
6.5488	6.78
3.2744	6.75
1.6372	6.73
0.8186	6.72
0.4093	6.66
0.2047	6.63
0.1023	6.60
0.0512	6.58
None	6.57

TABLE III

BACTERIA - NUTRIENT SYSTEMS STUDIED POLAROGRAPHICALLY*

Table Reference	Bacteria	Nutrient	Conditions
IV	*E. coli*	Brain Heart Infusion	Growth at 37°C
V-A	*E. coli*	Nutrient Broth	Growth at 37°C
V-B	*E. coli*	Nutrient Broth (with phosphate added)	Growth at 37°C
VI	*Desulfovibrio desulfuricans*	Liquid Starkey Medium	Growth at 24-25°C
VII-A	*Thiobacillus thioparus*	Sodium Thiosulfate ($Na_2S_2O_3$)	Metabilism at room temperature
VII-B	*Thiobacillus thioparus*	Sodium Sulfite (Na_2SO_3)	Metabolism at room temperature
VII-C	*Thiobacillus thioparus*	*Thiobacillus* Medium	Metabolism at room temperature
VIII	*E. coli* (concentrated by pre-growth)	Brain Heart Infusion	Growth at 37°C
IX	*E. coli* (concentrated by pre-growth)	Trypticase Soy Broth	Growth at 37°C.
X	Soil Sample #6	Trypticase Soy Broth	Growth at room temperature
XI	Soil Sample #9	Trypticase Soy Broth	Growth at room temperature

* Bacteria, nutrients and soils are described in Oxidation-Reduction Division.

TABLE IV

POLAROGRAPHIC ANALYSIS OF E. COLI - BRAIN HEART INFUSION AT 37°C

(Soln.)[1,2]	Incubation Time (hrs. at 37°C)	Anodic Half Wave Potential ($E_{1/2}$ vs. SCE)	Relative Diffusion Current (Wave Height)[3]	% Change in Diffusion Current (Wave height) due to Influence of *E. coli*
BHI Media (alone), polarogramed in 0.1M KNO_3	0	+0.26	1.00	reference
BHI Media, inoculated with *E. coli* polarogramed in 0.1M KNO_3[4]	4-1/2	+0.23	1.00	0
BHI Media, inoculated with *E. coli* polarogramed in 0.1M KNO_3[4]	19	+0.23	0.79	21
BHI Media (alone), polarogramed in H_2O	0	+0.36 +0.39	1.00 1.00	reference
BHI Media inoculated with *E. coli* polarogramed in H_2O[4]	4-1/2	+0.36 +0.39	1.00 1.00	0
BHI Media inoculated with *E. coli* polarogramed in H_2O[4]	19	+0.35 +0.39	0.81 0.79	19 21

(1) BHI is brain heart infusion media - made up as 101 gm. per liter of water (concentration of nutrients were then: Disodium phosphate, Na_2HPO_4, 7.5 gm/l.; sodium chloride, NaCl, 15 gm/l.; Bacto-Dextrose, 6 gm/l.; Proteose-Peptone (Difco), 30 gm/l.; beef heart (infusion from)750 gm ; calf brains (infusion from)600 gm .

(2) Samples were diluted 1/100 before polarograming; dilutions were with 0.1M KNO_3 solution or with water.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for BHI media (alone), diluted 1/100 before polarograming.

(4) Regular stored culture of E. coli inoculated in BHI media (1 to 500).

TABLE V-A

POLAROGRAPHIC ANALYSIS OF E. COLI - NUTRIENT BROTH

(Growth at 37°C)

(Soln.)(1,2)	Incubation Time (hrs. at 37°C)	Anodic Half Wave Potential ($E_{1/2}$ vs. SCE)	Relative Diffusion Current (Wave Height)(3)	% Change in Diffusion Current (Wave height) due to Influence of E. coli
Nutrient Broth Media (alone) polarogramed in 0.1M KNO_3	0	+0.28	1.00	reference
Nutrient Broth Media inoculated with E. coli, polarogramed in 0.1M KNO_3 (5)	18	+0.29	0.09	91
Nutrient Broth Media (alone) polarogramed in H_2O)	0	+0.34	1.00	reference
Nutrient Broth Media inoculated with E.coli, polarogramed in H_2O (5)	18	+0.34	See Footnote (4)	See Footnote (4)

(1) Nutrient Broth was made up as 8 gm. per liter of water (concentration of nutrient was then: Bacto-Beef Extract, 3 gm/l. and Bacto-Peptone, 5 gm./l.)

(2) Samples were diluted 1/100 before polarograming, dilutions in one case were with 0.1M KNO_3 solution and in the other case with water.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for Nutrient Broth media (alone), diluted 1/100 before polarograming.

(4) It was found that polarographic supporting electrolyte concentration was insufficient for satisfactory determination of anodic polarization curves when water was employed as diluent.

(5) Regular stored culture of E. coli inoculated in Nutrient Broth Media (1 to 500).

TABLE V-B

POLAROGRAPHIC ANALYSIS OF E. COLI - NUTRIENT BROTH - WITH PHOSPHATE ADDED

(Growth at 37°C)

(Soln.)(1,2)	Incubation Time (hrs. at 37°C)	Anodic Half Wave Potential ($E_{1/2}$ vs. SCE)	Relative Diffusion Current (Wave Height)(3)	% Change in Diffusion Current (Wave height) due to Influence of E. coli
Nutrient Broth plus phosphate as media polarogramed in 0.1M KNO_3	0	+0.34	1.00	reference
Nutrient Broth plus phosphate inoculated with E. coli, polarogramed in 0.1M KNO_3 (5)	18	+0.34	1.00 See Footnote (4)	0
Nutrient Broth plus phosphate as media polarogramed in H_2O	0	+0.43	1.00	reference
Nutrient Broth plus phosphate as media inoculated with E. coli polarogramed in H_2O (5)	18	+0.43	1.00 See Footnote (4)	0

(1) Media was Nutrient Broth made up as 8 gm. per liter of water (concentrations of nutrient were: Bacto-Beef Extract, 3 gm/l. and Bacto-Peptone, 5 gm./l.). Also added the following phosphates to the media: Disodium hydrogen phosphate, Na_2HPO_4, 3.55 gm/l., sodium dihydrogen phosphate, NaH_2PO_4, 3.00 gm./l.

(2) Samples were diluted 1/100 before polarograming, dilutions in one case were with 0.1M KNO_3 solution and in the other case with water.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for Nutrient Broth plus phosphate media, diluted 1/100 before polarograming.

(4) Addition of phosphate to media apparently enhances anodic polarization wave. Minor composite wave observed previously (See Table V-A) is now blanketed by large phosphate wave and thus previously observed diminution caused by E. coli is no longer observable. Diffusion current (wave height) being same with and without E. coli when polarogramed in KNO_3 or water seems to indicate that phosphate ion is not consumed by E. coli during 18 hour incubation.

(5) Regular stored culture of E. coli inoculated in media (1 to 500).

TABLE VI

POLAROGRAPHIC ANALYSIS OF DESULFOVIBRIO DESULFURICANS - LIQUID STARKEY

(Growth at 24-25°C)

(Soln.)(1,2)	Incubation Time (hrs.)	Anodic Half Wave Potential ($E_{1/2}$ vs. SCE)	Relative Diffusion Current (Wave Height)(3)	% Change in Diffusion Current (Wave height) due to Influence of E. coli
Liquid Starkey as media polarogramed in 0.1M KNO_3	0	+0.26	1.00	reference
Liquid Starkey as media inoculated with Desulfovibrio desulfuricans polarogramed in 0.1M KNO_3	5	+0.26	0.10	90
Liquid Starkey as media inoculated with Desulfovibrio desulfuricans polarogramed in 0.1M KNO_3	18	+0.26	0.06	94

(1) Media was Starkey (liquid) of the following composition per liter: Peptone 2.5 gm., beef extract 1.5 gm., yeast extract 0.1 gm., magnesium sulfate ($MgSO_4 \cdot 7H_2O$) 1.5 gm., sodium sulfate (Na_2SO_4) 0.75 gm., ferrous ammonium sulfate 0.1 gm., tap water 500 ml., glucose (dextrose) 2.5 gm.

(2) Samples were diluted 1/100 with 0.1M KNO_3 solution before polarograming.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for liquid Starkey media, diluted 1/100 before polarograming.

TABLE VII-A

POLAROGRAPHIC ANALYSIS OF THIOBACILLUS THIOPARUS - SODIUM THIOSULFATE SOLUTION (METABOLISM AT ROOM TEMPERATURE)

(Soln.)[1,2]	Shaking Time (hrs. at room temperature)	Anodic Half Wave Potential ($E_{1/2}$ vs. SCE)	Relative Diffusion Current (Wave Height)[3]	% Change in Diffusion Current (Wave Height) Due to Influence of *Thiobacillus thioparus*
Sodium Thiosulfate ($Na_2S_2O_3$) solution (alone) polarogramed in 0.1M KNO_3[5]	0	None	None	---
Thiobacillus thioparus Concentrate dispersed in water[4]	3	+0.56	1.00	reference
Thiobacillus thioparus Concentrate dispersed in sodium thiosulfate solution	3	+0.56	0.875	12.5
Thiobacillus thioparus Concentrate dispersed in sodium thiosulfate solution	75	+0.56	0.750	25.0

- Limiting percentage change decrement detectable -- 100.0

(1) Medium was 7 gm. of sodium thiosulfate ($Na_2S_2O_3$) per liter of solution.

(2) Samples were diluted 1/100 with 0.1M KNO_3 solution before polarograming.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for Thiobacillus thioparus concentrate dispersed in water, diluted 1/100 before polarograming. Organism in water alone, after initial 3 hour growth period, is taken as comparative standard here since occluded anions from heavy inoculum apparently form the composite anodic polarization wave.

(4) Thiobacillus thioparus concentrate was prepared as follows:

(a) Grew Thiobacillus thioparus concentrate in Thiobacillus Media (see ingredients listed below) for four days at 25°C. pH was adjusted to neutrality with 10% $NaHCO_3$ twice a day.

(b) Harvested Thiobacillus thioparus by spinning down whole mass from about 1 liter of (a), washed mass twice with sterile H_2O. Suspended the whole mass from this operation in 15 ml. of sterile H_2O. This Thiobacillus thioparus concentrate was then used as the inoculum in all the anodic polarization studies.

(c) Inoculum (Thiobacillus thioparus concentrate) was diluted 1/10 in each media studied (i.e., in water and in sodium thiosulfate solution).

(5) $Na_2S_2O_3$ media as given in footnote above was diluted 1/10 with water here before diluting 1/100 with 0.1M KNO_3 for polarograming. Reason was to adjust to same concentration of thiosulfate as present in other polarograms (i.e., to adjust for 1/10 dilution when organism is added in other comparison determinations).

(6) Since sodium thiosulfate ($Na_2S_2O_3$) alone shows no interfering anodic wave, then limit of detection of percent change is 100%.

TABLE VII-B

POLAROGRAPHIC ANALYSIS OF THIOBACILLUS THIOPARUS - SODIUM SULFITE SOLUTION

(METABOLISM AT ROOM TEMPERATURE)

(Soln.)[1,2]	Shaking Time (hrs. at room temperature	Anodic Half Wave Potential ($E_{1/2}$ vs. SCE)	Relative Diffusion Current (Wave Height)[3]	% Change in Diffusion Current (Wave Height) due to Influence of *Thiobacillus thioparus*
Sodium sulfite (Na_2SO_3) solution (alone) polarogramed in 0.1M KNO_3[5]	0	+0.56	0.75	--
Thiobacillus thioparus concentrate dispersed in water[4]	3	+0.56	1.00	reference
Thiobacillus thioparus concentrate dispersed in sodium sulfite solution	3	+0.56	0.875	12.5
Thiobacillus thioparus concentrate dispersed in sodium sulfite solution	72	+0.56	0.750	25.0
(Limiting percentage change decrement detectable)[6]			-	25.0

(1) Medium was 7 gm. of sodium sulfite (Na_2SO_3), per liter of solution.

(2) Sample were diluted 1/100 with 0.1M KNO_3 solution before polarograming.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for Thiobacillus thioparus concentrate dispersed in water, diluted 1/100 before polarograming. Organism in water alone, after initial 3 hour growth period, is taken as comparative standard here since occluded anions from heavy inoculum apparently form the composite anodic polarization wave.

(4) Thiobacillus thioparus concentrate was prepared as follows:

(a) Grew Thiobacillus thioparus concentrate in Thiobacillus Media (see ingredients listed below) for four days at 25°C. pH was adjusted to neutrality with 10% $NaHCO_3$ twice a day.

(b) Harvested Thiobacillus thioparus by spinning down whole mass from about 1 liter of (a), washed mass twice with sterile H_2O. Suspended the whole mass from this operation in 15 ml. of sterile H_2O. This Thiobacillus thioparus concentrate was then used as the inoculum in all the anodic polarization studies.

(c) Inoculum (Thiobacillus thioparus concentrate) was diluted 1/10 in each media studied (i.e., in water and in sodium sulfite solution).

(5) Na_2SO_3 media as given in footnote above was diluted 1/10 with water here before diluting 1/100 with 0.1M KNO_3 for polarograming. Reason was to adjust to same concentration of sulfite as present in other polarograms (i.e., to adjust for 1/10 dilution when organism is added in other comparison determinations).

(6) Since sodium sulfite (Na_2SO_3) alone shows an anodic wave which is 75% as high as reference wave at same $E_{1/2}$, then percentage changes greater than 25% are not detectable in this media.

TABLE VII-C

POLAROGRAPHIC ANALYSIS OF THIOBACILLUS THIOPARUS- THIOBACILLUS MEDIA (TBM)

(METABOLISM AT ROOM TEMPERATURE)

(Soln.)[1,2]	Shaking Time (hrs. at room temperature)	Anodic Half Wave Potential ($E_{1/2}$ vs. SCE)	Relative Diffusion Current (Wave Height)[3]	% Change in Diffusion Current (Wave Height) due to Influence of Thiobacillus thioparus
Thiobacillus Media, TBM, alone) polarogramed in 0.1M KNO_3[5]	0	+0.56	1.00	reference
Thiobacillus thioparus concentrate dispersed in Thiobacillus Media (TBM)[4]	3	+0.56	0.813	18.7
Thiobacillus thioparus concentrate dispersed in Thiobacillus Media (TBM)	72	+0.56	0.688	31.2

(1) Media was Thiobacillus Media of the following composition per liter:

$Na_2S_2O_3 \cdot 5H_2O$	10 gm.
NH_4Cl	1 gm.
$MgCl_2.6H_2O$	0.5 gm.
KH_2PO_4	0.4 gm.
K_2HPO_4	0.6 gm.
$FeCl_3$	0.020 gm.

(2) Samples were diluted 1/100 with 0.1M KNO_3 solution before polarograming.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for Thiobacillus thioparus Media (TBM), diluted 1/100 before polarograming.

(4) Thiobacillus thioparus concentrate was prepared as follows:

(a) Grew Thiobacillus thioparus concentrate in Thiobacillus Media (see ingredients listed above) for four days at 25°C. pH was adjusted to neutrality with 10% $NaHCO_3$ twice a day.

(b) Harvested Thiobacillus thioparus by spinning down whole mass from about 1 liter of (a), washed mass twice with sterile H_2O. Suspended the whole mass from this operation in 15 ml. of sterile H_2O. This Thiobacillus thioparus concentrate was then used as the inoculum in all the anodic polarization studies.

(c) Inoculum (Thiobacillus thioparus concentrate) was diluted 1/10 with Thiobacillus Media (TBM).

(5) Thiobacillus Media (TBM) as given in footnote above was diluted 1/10 with water here before diluting 1/100 with 0.1M KNO_3 for polarograming. Reason was to adjust to same concentration of nutrient constituents present in other polarograms (i.e., to adjust for 1/10 dilution when organism is added in other comparison determinations).

TABLE VIII

COMPARATIVE ANALYSIS OF E. COLI IN BRAIN HEART INFUSION MEDIA

Incubation Time (Hrs. at 37°C) (4)	Anodic Half Wave Potential $E_{1/2}$ vs. SCE (2)	Relative Diffusion Current (Wave Height) (3)	% Change in Diffusion Current (Wave Height) due to Influence of *E. coli*	Coulter Counts	pH
BHI Blank (no *E. coli*) (1)	+0.28	1.00	reference	--	7.38
0.00	+0.28	1.00	0	1.82×10^7	7.38
0.50	+0.28	0.93	7.0	1.86×10^7	7.38
1.50	+0.28	0.81	19.0	3.63×10^7	7.33
2.50	+0.28	0.73	27.0	1.55×10^8	6.20
3.50	+0.28	0.78	22.0	1.47×10^9	6.37
5.00	+0.28	0.94	6.0	3.27×10^9	6.45
21.00	+0.29	0.98	2.0	3.33×10^9	7.14

(1) BHI is brain heart infusion media - made up as 37 gm per liter of water (concentration of nutrients were then: Disodium phosphate, Na_2HPO_4, 2.5 gm/l; sodium chloride, NaCl, 5 gm./l; Bacto-Dextrose, 2 gm/l; Proteose-Peptone (Difco), 10 gm/l; Beef Heart (infusion from) 250 gm ; Calf Brains (infusion from) 200 gm).

(2) Samples were diluted 1/100 with 0.1M KNO_3 solution before polarograming.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for BHI Media (alone), diluted 1/100 before polarograming.

(4) *E. coli* inoculum was a concentrated (18 hr. freshly grown culture) dilution into BHI media (1 to 100).

TABLE IX

COMPARATIVE ANALYSIS OF E. COLI IN TRYPTICASE SOY BROTH MEDIA

Incubation Time (Hrs. at 37°C) (4)	Anodic Half Wave Potential $E_{1/2}$ vs. SCE (2)	Relative Diffusion Current (Wave Height) (3)	% Change in Diffusion Current (Wave Height) due to Influence of E. coli	Coulter Counts	pH
TSB Blank (No E. coli)	+0.28	1.00	reference	--	7.33
0.00	+0.28	1.00	0	$1.36X10^7$	7.31
0.50	+0.28	0.94	6.0	$2.24X10^7$	7.17
1.50	+0.27	0.83	17.0	$2.13X10^8$	6.37
2.50	+0.28	0.73	27.0	$4.13X10^8$	5.77
3.50	+0.27	0.78	22.0	$1.20X10^9$	5.83
5.00	+0.28	0.94	6.0	$3.23X10^9$	6.00
21.00	+0.27	0.98	2.0	$3.74X10^9$	6.88

(1) TSB is Trypticase Soy Broth Media - made up as 30 gm. per liter of water. Concentration of nutrients were then: Sodium chloride, NaCl, 5.0 gm/l; potassium phosphate, K_2HPO_4, 2.5 gm/l; dextrose, 2.5 gm/l; Trypticase (Pancreatic digest of casein), 17.0 gm/l; and Phytone (soy peptone), 3.0 gm/l.

(2) Samples were diluted 1/100 with 0.1M KNO_3 solution before polarographing.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for TSB media (alone), diluted 1/100 before polarographing.

(4) E. coli inoculum was a concentrated (18 hr. freshly grown culture), dilution into TSB media (1 to 100).

TABLE X

SIMULTANEOUS POLAROGRAPHIC ANALYSIS WITH REDOX VALUES ON SOIL SAMPLE #6 IN TRYPTICASE SOY BROTH (1)

Growth Time (Hrs.) at Room Temp. (4)	Anodic Half Wave Potential $E_{1/2}$ vs SCE (2)	Relative Diffusion Current (Wave Height) (3)	% Change in Diffusion Current (Wave Height) due to Influence of Soil	Redox Voltage vs NHE
0	+0.55	1.00	0	+0.361
0.5	+0.55	0.94	6.0	+0.361
1.0	+0.55	0.84	16.0	+0.361
2.5	+0.55	0.80	20.0	+0.356
18.5	+0.55	0.74	26.0	+0.230
23.5	+0.55	0.44	56.0	+0.200
42.5	+0.55	0.41	59.0	-0.160
50.5	+0.55	0.40	60.0	-0.170

(1) TSB is trypticase soy broth - made up as described in previous table.

(2) Samples were diluted 1/100 with 0.1M KNO_3 solution before polarograming.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for TSB (alone) just after soil added, diluted 1/100 before polarograming.

(4) Soil #6 was described previously, 2.83 gms. were added to 850 ml of TSB nutrient solution (approximating usual 0.3% concentration Redox Experiments). Polarographic analyses were made on small aliquot samples removed aseptically.

TABLE XI

SIMULTANEOUS POLAROGRAPHIC ANALYSIS WITH REDOX VALUES ON SOIL SAMPLE #9 IN TRYPTICASE SOY BROTH (1)

Growth Time (Hrs.) at Room Temp. (4)	Anodic Half Wave Potential $E_{1/2}$ vs SCE (2)	Relative Diffusion Current (Wave Height) (3)	% Change in Diffusion Current (Wave Height) due to Influence of Soil	Redox Voltage vs NHE
0	+0.55	1.00	0	+0.396
0.5	+0.55	0.96	4.0	+0.396
1.0	+0.55	0.88	12.0	+0.396
2.5	+0.55	0.83	17.0	+0.396
18.5	+0.55	0.77	23.0	+0.200
23.5	+0.55	0.52	48.0	+0.170
42.5	+0.55	0.48	52.0	+0.030
50.5	+0.55	0.47	53.0	+0.025

(1) TSB is trypticase soy broth - made up as described in previous table.

(2) Samples were diluted 1/100 with 0.1M KNO_3 solution before polarograming.

(3) Diffusion current (wave height) of anodic polarization wave is arbitrarily taken as 1.00 for TSB (alone) just after soil added, diluted 1/100 before polarograming.

(4) Soil #9 was described previously, 2.83 gms. were added to 850 ml of TSB nutrient solution approximating usual 0.3% concentration in Redox experiments. Polarographic analyses were made on small aliquot samples removed aseptically.

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